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(Part II appears in the January–February 1977 issue of The Mount Sinai Journal of Medicine.)

Mount Sinai's Patients: Trends and Prospects

CARTER L. MARSHALL, M.D.

The purposes of this report are three: to describe the patient population of the Mount Sinai Hospital, to identify trends and changes in this population over time, and to point out in a most tentative way what these trends and changes portend as the hospital looks toward the advent of National Health Insurance (NHI).

Sources

Perhaps the most complete single source of patient information is the report "Development of an Automated Patient Identification and Statistics System" prepared by Dr. Margaret Olendzki in 1969 (1). Dr. Olendzki was then Assistant Professor of Community Medicine and her work focuses on the year 1967. The data on which the report is based is now nearly eight years old but proved valuable in providing a baseline against which more recent data, especially for outpatients, could be compared. It is also valuable as a guide to the kinds of data the hospital would need should it decide to establish a continuing effort in long-range planning.

The most useful source of inpatient data is the March 10, 1971 Blue Cross Census Summary Report which described Mount Sinai patients on that date and included as well data from similar one-day census studies in 1961, 1964, and 1967 (2). While this report thus covers a ten-year period, the extent to which such cross-sectional studies describe an entire year's patient experience is questionable and conclusions based on these data should be viewed with some skepticism. The Blue Cross report was to be updated to include a day in 1974 but this information is not presently available. Beginning in 1966, Mount Sinai began automating patient data. There are various inconsistencies in the early automated data which make them difficult to use, and they were not used in preparing this report. Reliable outpatient data begin in 1971 and I am indebted to Mr. Patrick Kilte of the Social Service Department for his help in their compilation.

There are, then, three principal sources for this report. The Blue Cross Census Summary for inpatients, the data from Social Service for outpatients, and the Olendzki report which provides a useful baseline on all patients in the late 1960's.

No one knows what the precise impact of NHI will be on this hospital

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although nearly everyone would agree that it will be massive and lead to changes of major import. This imponderable, plus the recent and rapid rise of suburban hospitals as serious competitors to urban medical centers, makes the use of past data a less reliable guide to the future than might otherwise be the case. In addition, the impact of Professional Standards Review Organizations (PSRO) is an unknown quantity which demands close scrutiny. Another imponderable is the potential effect of the establishment of a family practice or primary care residency program at the medical center. One reasonable expectation of such a residency would be an increase in the proportion of inpatients from the South Bronx and East Harlem. The extent of this increase would be reflected in a possible shift of inpatient characteristics and illness patterns away from those of a regional referral center toward those of a community hospital. Thus, NHI, suburban competition, PSRO, and a new residency represent developments whose influence on Mount Sinai will no doubt be great. While we cannot predict the nature of this influence with assurance, we can expect with some confidence that the forces that will shape our future will not be those that shaped our past, and trends identified from secular data may well be more apparent than real. For this reason the limitations of existing data are less important today than they would have been five years ago.

Inpatients

Between 1961 and 1971 there was little change in the sex or marital status of Mount Sinai patients. About 58% were female both in 1961 and in 1971, and there is no consistent trend in the ratio of the married, single, separated, or widowed, to each other. Interesting changes have occurred, however, with respect to age, race, and place of residence. These data appear in Table I and show that the inpatient population is shifting increasingly toward older and browner patients. The under-12 pediatric population has dropped steadily since 1961 while the adolescent group had increased 50% by 1971. The geriatric age group has increased markedly, stimulated by Medicare, from 24.4% of the patient population in 1961 to an estimated 34.3% in 1974. These trends reflect the declining birth rate and the aging of the population. Meanwhile, the proportion of nonwhite patients is up 45%, reflecting the changing demographic composition of the city and perhaps the advent of Medicaid as well.

The proportion of patients from New York City has declined almost 10%, most of this a result of a decline in patients from Manhattan. Changes in the other boroughs have been minor. The City's decline is attributable to modest increases from Westchester and Nassau-Suffolk and a close to 100% increase in patients from New Jersey and Connecticut. Much of the increase from these two neighboring states is doubtless attributable to the liberal abortion law enacted by New York in 1970.

Table II shows how long people stay in the hospital, how their bills are paid, and what kinds of rooms they occupy. During the decade 1961–1971, length of

TABLE I

*Selected Characteristics of Mount Sinai Inpatients on Specified Dates in 1961, 1964, 1967, 1971**

	946 5/10/61	1094 10/20/64	1084 11/9/67	1103 3/10/71	Other Voluntary Hospitals 1971	Estimate** 1974
Age						
<12 Years	10.8%	10.6%	9.0%	8.1%	8.9%	6.8%
13–19	3.9	4.6	4.9	5.7	4.4	6.3
20–34	18.6	20.2	18.0	22.0	17.5	
35–64	41.9	41.1	38.9	39.8	38.2	
65+	24.4	21.6	27.4	30.2	29.9	34.3
No Information	0.4	1.9	0.8	0.2	1.1	
Race						
White	86.4	85.4	85.2	82.5	77.6	80.9
Non-White	13.2	12.9	14.8	17.0	15.0	19.1
Residence						
New York City	86.8	86.4	85.7	81.6		79.2
Manhattan***	47.8 (55%)	43.9 (51%)	44.9 (51%)	40.1 (49%)		
Bronx	16.4 (19%)	16.0 (19%)	15.8 (18%)	16.5 (20%)		
Brooklyn	10.3 (12%)	12.2 (14%)	10.4 (12%)	11.1 (14%)		
Queens-Richmond	12.3 (14%)	14.3 (17%)	15.5 (18%)	13.9 (17%)		
Westchester	2.3	1.6	2.2	3.2		
Nassau-Suffolk	3.9	3.4	3.3	4.1		
New Jersey-Conn.	3.6	5.4	4.8	7.1		
New York State	1.8	1.2	1.8	1.9		
All Other	1.7	1.2	1.2	1.4		
Unknown	0.3	0.1	0.2	—		

* Columns may not add to 100% because of rounding.

** Estimates for 1974 based in average increase or decrease of the two three-year periods 1964–67 and 1967–71 applied to 1971.

*** Figures in parentheses indicate proportion of total New York City patient population from each borough.

Source: Modified from Blue Cross Census Summary, 1971.

stay gradually diminished but was still slightly higher than the average of other voluntary hospitals. With regard to method of payment, the big factor is the Social Security Act of 1965. Medicaid/Medicare covered almost half of all payments by 1971 as the proportions provided by Blue Cross, commercial carriers and self-payment declined during the ten-year period. Perhaps the most spectacular change has been the social security transformation of yesterday's ward patient into today's semi-private patient. This transformation is more pronounced at Mount Sinai than in other voluntary hospitals. The reasons for this are unclear.

Outpatients

As mentioned earlier, outpatient department (OPD) data is of little use prior to 1971 with the exception of some of the Olendzki data for the year 1967. With the exception of the year of the strike, 1973, the trend in OPD visits has been ever upward: 201,000 in 1970, 206,000 in 1971, 214,000 in 1972, and

TABLE II

Length of Stay, Method of Payment, and Type of Accomodation of Mount Sinai Patients 1961, 1964

	5/10/61	10/20/64	11/9/67	3/10/71	Other Voluntary Hospitals
Length of stay					
*Cumulative %**					
<8 days	NA	15.6%	19.0	17.4	19.4
<15 days	—	32.7 (17.1)	35.2 (16.2)	36.6 (19.2)	39.2 (19.8)
<22 days	—	47.0 (14.3)	50.2 (15.0)	51.7 (15.1)	54.2 (15.0)
22 + days	—	100.0 (53.0)	100.0 (49.8)	100.0 (48.3)	100.0 (45.8)
Method of Payment					
Blue Cross	54.8	55.9	43.9	35.5	36.3
Medicare			27.1	30.2	29.3
Welfare/Medicaid	23.4	20.7	18.7	17.4	18.5
Commercial insur	7.8	11.3	6.0	6.3	10.4
Self-Payment	13.5	11.2	3.0	6.8	3.6
Other	0.6	0.9	1.3	3.5	1.3
Accomodation					
Private	18.7	16.4	19.9	14.9	13.1
Semi-private	32.2	37.4	40.3	65.3	53.1
Ward	38.5	39.3	30.1	8.0	23.5
Other**	10.5	6.9	9.7	11.3	10.1

* Figures in parenthesis are the %'s of that group, figures not in parenthesis are cumulative.

** Pediatrics, nursery, prematures.

Source: Blue Cross Census Summary, March 10, 1971.

211,000 in 1973. The ten most heavily used clinics accounted for 30% of all OPD visits in 1971 and 39% in 1973. For both years, there were 2.8 visits per patient. In 1973, 57,781 patients made 87,222 emergency room (ER) visits or about 1.5 visits per patient. During the first nine months of 1971, 50,963 patients made 77,271 ER visits, again averaging 1.5 visits per patient. Clinic visits are covered principally by Medicare and/or Medicaid which paid for 50% of all visits in 1973 and about 53% in 1971. Self-pay accounted for the bulk of the remainder, 44% in 1973 and 43% in 1971. Although self-pay accounted for almost half of all visits, this source of payment guaranteed only 13% of clinic income in 1971 while Medicaid/Medicare provided the remaining 87%.

Tables III and IV deal with patient utilization of the OPD by visits and by clinics. These tables show little change between 1967 and 1971 and they suggest, as Olendzki pointed out in 1969, that "the general patient load picture at Mount Sinai is one of constant flux and turnover." While about 40% of patients make a single visit, a relative handful of patients (10%) account for about 40% of all visits. Table IV shows that nearly 80% of all patients use one or two clinics only. The similarity between the 1967 and 1971 data in Tables III and IV suggest that Table V, which is taken directly from the Olendzki report, is still relevant. It shows that from a large group of patients the first month seen is also the last month seen, indicative of episodic use of the OPD by most patients. This pattern continued into 1973. In that year, about half of all clinic patients seen were new to the clinics; and of these new patients, 75% made their first Mount Sinai contact in the ER; while 41% of patients made a single visit in that year, up only slightly from 1967 or 1971.

Table VI shows the geographic distribution of patients registered with the OPD in 1967, 1971, and 1973. Table VII shows the distribution of patients within Manhattan. These tables repeat the inpatient pattern in that the hospital catchment is primarily Manhattan and the Bronx. However, inpatients are predominantly white and outpatients are predominantly black and Puerto Rican. This latter observation is suggested by the heavy concentration of outpatients from East Harlem and the fact that 61% of outpatients from the Bronx came from the South Bronx in 1971. Direct ethnic data is not available for outpatients.

The more important trends both among inpatients and outpatients are summarized in Table VIII. It is worth emphasizing here that without any question whatever the most significant change is the increasing dependence of the hospital on federal and state funding of patient services. To a real extent, Mount Sinai along with other hospitals is vassal to the federal overlord.

TABLE III

Percentage Distribution of Patients and visits, by Number of Visits per Patient, 1967 and 1971

Number of Visits	1967		1971	
	Patients	Visits	Patients	Visits
1	39.3	9.2	39.9	10.2
2	16.4	7.7	16.8	8.6
3	9.9	6.9	10.4	7.9
4	6.9	6.5	7.1	7.2
5	5.0	5.8	5.1	6.6
6–9	11.3	19.4	10.9	19.9
10+	11.2	44.5	9.8	39.6

TABLE IV

Distribution of Patients by Number of Clinics Used, 1967 and 1971

Numbers of Clinics	% of Total Patients	
	1967	1971
1	58.9	54.5
2	21.1	24.1
3	9.9	11.0
4	4.8	5.5
5	2.4	2.6
6	1.3	1.2
7	.7	.6
8	.4	.3
9	.2	.1
10+	.3	.1

TABLE V

Duration of Episode of Care for Each Patient, within the Year 1967

Patient Last Seen	Patient First Seen												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
January	2,808												2,808
February	515	2,137											2,652
March	514	378	2,325										3,217
April	484	274	390	2,377									3,525
May	441	297	298	383	2,426								3,845
June	463	264	258	253	437	2,161							3,836
July	415	217	222	202	213	348	2,357						3,974
August	422	253	248	225	226	221	371	2,498					4,464
September	534	310	331	255	229	241	233	398	2,333				4,864
October	707	384	348	377	282	232	238	263	425	2,622			5,878
November	1,172	589	474	389	363	324	292	344	365	587	2,670		7,569
December	2,994	1,451	888	621	642	543	515	601	621	784	997	3,912	14,569
Total	11,469	6,554	5,782	5,082	4,818	4,070	4,006	4,104	3,744	3,993	3,667	3,912	61,201

Source: Olendzki.

TABLE VI

Geographic Distribution of Patients Registered with the Mount Sinai Hospital OPD 1967, 1971, 1973

Residence	% of Patients		
	1967	1971	1973
Manhattan	64%	62%	60%
Bronx	24%	25%	26%
Brooklyn	5%	5%	5%
Queens	3%	2%	5%
Other	4%	6%	4%
	100%	100%	100%

Sources: Olendzki and Social Service Department.

Conclusion

There is nothing surprising in Table VIII. All this report does is to present in summary what is already known to many in the Mount Sinai community. In my opinion more recent and more complete hospital data would add little to what we already know. Some of the trends will no doubt continue, such as the aging and "browning" of the inpatient population, the use of the ER and OPD as a primary care resource for East Harlem and the South Bronx, and the firmly established hegemony of the federal and state governments. As mentioned at the beginning of this report, federal involvement through programs such as NHI, PSRO, HMO, Medicaid, and Medicare make the future difficult to predict. This difficulty briefly can be illustrated by a consideration of National Health Insurance. Newhouse et al have attempted to predict changes in demand for inpatient and ambulatory services resulting from various forms of NHI (3). They point out that while the increase in demand for inpatient services will be comparatively modest, demand for ambulatory care will vary from a low of 0% among those whose existing coverage is equivalent to what NHI might offer to a high of 120% among people for whom NHI would provide full coverage for the first time. In New York City third-party coverage of indigent patients is far better than the national norm. Does this mean that NHI-induced increase in demand for ambulatory care would be minimal? Not necessarily, since the city also contains hundreds of thousands of marginal middle-income people who would doubtless increase their consumption of ambulatory care were it offered free or at a modest level of coinsurance. On the other hand, about 86% of city physicians will not accept Medicaid patients. A system of NHI which opened up this inaccessible pool of practitioners might actually reduce the demand on the hospital's OPD. Sparer and Anderson examined the utilization rate of low-income families receiving

TABLE VII

Distribution of Registered Mount Sinai Outpatients from Manhattan, 1967 and 1971

Area	% of Patients	
	1967	1971
South of W. 59th & 96th Sts.	9	10
W. 60th St. to W. 133rd St.	19	17
North of W. 134th St. & E. 130 St.	8	12
E. 97th St. to E. 129th St.	64	62

Sources: Olendzki and Social Service Department.

TABLE VIII

Trends among Inpatients and Outpatients, Mount Sinai Hospital, Various Years, 1961 to 1973

Inpatients Trends

1) Higher proportion of older patients
2) Higher proportion of nonwhite patients
3) Declining proportion of patients from Manhattan with a slight increase in patients from the other boroughs, Westchester, Nassau, and Suffolk. Spectacular increase in patients from New Jersey and Connecticut is considered temporary due to New York's early action liberalizing its abortion law.
4) Slight decline in length of stay in hospital.
5) Increasing dependence on Medicaid/Medicare for patient expenses.
6) Spectacular rise in proportion of semi-private patients at the expense of ward services.

Outpatient Trends

1) Increase in total visits each year.
2) Increasing dependence on Medicaid/Medicare from patient expenses.
3) Patients tend to use OPD on an episodic, noncontinuous basis except from a relatively few "higher utilizers."
4) Patients come overwhelmingly from East Harlem and the South Bronx.
5) Patients are predominantly black, Puerto Rican, and poor.
6) The ER is the predominant route into the clinic system.

coverage through prepaid group practice for the first time and found that utilization was initially higher for about six months, by which time it stabilized at a level comparable to regular subscribers (4). Meanwhile, McDonald et al found that the introduction of health insurance in Canada did not produce a flood of visits for trivial complaints (5). What does all this mean for our hospital? Specific effects on our patient population are unknown, but it is my belief that the forces generating these changes are quite clear.

None of the state or federal initiatives arose within the medical profession. Rather they have emerged in response to rising consumerism and represent Congressional attempts to make quality care available at an affordable cost. The dual and perhaps conflicting concerns of quality and cost are the basis of all federal initiatives and the rising interest in basing quality assessment on outcome measures stressing function and prognosis can only serve to underscore the limitations of the medical care process. Over the past two decades, there has been little or no increase in life expectancy in the United States and additional expenditures in health care will not, in all likelihood, alter this state of affairs. Social and behavioral factors which increase the risk of premature death, such as poverty, alcoholism, smoking, obesity, lack of exercise, and auto accidents, lie largely beyond the boundaries of existing health services. At the same time, therapy for cardiovascular diseases and cancer leaves much to be desired, and preventive measures such as annual physicals and multiphasic screening seem to have little effect on longevity. The highest return on health dollars would come from successful education of the public leading to lifestyle modifications which would reduce the risk of premature death. Eventually, some of the resources now flowing into health services will probably be diverted into the area of lifestyle modification.

It is my recommendation that the medical center consider how it can begin educating its inpatients and above all its low-income ambulatory care constituency which represents what seems to be a permanent drain of our resources. It is also recommended that we consider the development of OPD satellites remote from the hospital, staffed by ancillary personnel, and perhaps supported by a telecommunication link to hospital-based physician back-up. This form of outreach would probably reduce visits to the OPD while providing equivalent care at lower cost in a setting which might be designed to facilitate the development of an informed patient. My final recommendation is that, having labored over a century to develop Mount Sinai as a center of medical excellence, the medical center should now consider ways that it can involve the patient and the potential patient communities in the promotion and maintenance of their own health. This seems the surest approach to cost containment and quality assessment that will stand up under outcome scrutiny.

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Myasthenia Gravis Unmasked by Antiarrhythmic Agents*

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Abstract

Use of quinidine and procaine amide should be avoided in patients with myasthenia gravis because of their depressant effects on neuromuscular conduction. Two case reports illustrate how these antiarrhythmic agents can unmask previously unsuspected myasthenia gravis and exacerbate previously well-controlled symptoms. The pharmacologic mechanisms of drug action are reviewed and suggestion for use of alternate antiarrhythmic agents are made.

Quinidine and procaine amide are among the most widely used drugs to counter cardiac arrhythmias. While both agents primarily affect cardiac excitability and conduction and increase the atrial refractory period, quinidine also depresses cardiac contractility. In addition, both drugs have anticholinergic properties which affect neuromuscular conduction. However, these toxic side-effects are only rarely observed clinically. We wish to present two case reports wherein these compounds unmasked clinical myasthenia gravis previously unsuspected in one instance and caused exacerbation of previously well-controlled myasthenia gravis in another.

Case Reports

Case No. 1: WB, a 67-year-old executive had suffered from moderate hypertension for the past ten years. He was treated with sodium restriction and hydrochlorothiazide. At age 64 the patient suffered an acute anteroseptal myocardial infarction from which he recovered uneventfully. A year later, while asymptomatic, the electrocardiagram revealed multifocal premature ventricular contractions which responded promptly to 0.2 gm quinidine orally four times daily. Within 72 hours of initiation of this therapy the patient began to complain of diplopia on left lateral gaze, but denied any other neurologic symptoms. At this time the patient recalled that he had experienced three brief transient attacks of similar diplopia on left lateral gaze, never lasting more than five minutes and usually associated with exposure to bright sunlight, during the last 20 years. Complete neurologic examination revealed only left lateral rectus paresis which did not respond to intravenous placebo or 3 mg edrophonium, but responded to 10 mgm edrophonium intravenously. Serum electrolytes were within normal range. Electromyographic studies, including repetitive supramaximal percutaneous nerve stimulation on left forearm, were normal, but a regional curare test was definitely positive (1). Tomography of the lateral mediastinum was negative for thymoma. Quinidine was withdrawn and diplopia disappeared completely 48 hours later. Because of recurrence of ventricular and nodal premature contractions unresponsive to propanolol the patient was started on procaine amide 500 mg every six hours. The arrhythmia disappeared promptly but the diplopia

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recurred on the fourth day of procaine amide therapy. Edrophonium testing was positive only when 10 mg was used. The procaine amide dosage was reduced to 250 mg every six hours without change in diplopia and oral pyridostigmine 30 mgm every four hours was added. This decreased the diplopia considerably, but never completely. Over the next three months procaine amide was gradually eliminated without recurrence of arrhythmia. Diplopia and need for anticholinesterase medication also disappeared after cessation of antiarrhythmia therapy. Presently the patient is asymptomatic, but diplopia on left lateral gaze can be produced by prolonged gazing into bright light.

Case No. 2: JK is a retired 76-year-old telephone repairman with a 12-year history of coronary insufficiency and congestive heart failure which responded to digitalization and intermittent diuretic therapy. At the age of 68 the patient developed ptosis, dysphagia, weakness of the neck and of the shoulder and hip girdles. Edrophonium test was strongly positive as was repetitive supramaximal percutaneous nerve stimulation on left forearm. Routine chest x-rays and tomography of the lateral mediastinum showed the anterior mediastinum to be free of any masses. Myasthenia gravis was well controlled by oral pyridostigmine 60–75 mg on a four-hourly basis. On this regimen the patient remained essentially asymptomatic for three and a half years. Suddenly without obvious cause and without clinical signs of overdosage, all previous myasthenia symptoms recurred with extreme severity. The patient was no longer able to ambulate, had difficulty holding up his head or keeping his mouth closed and experienced nasal regurgitation when trying to swallow. Edrophonium test, using 2–10 mg intravenously, one hour after last dose of pyridostigmine was negative and the previous anticholinesterase drug dose was therefore left unchanged. About ten days prior to exacerbation of myasthenic symptoms, the patient had contacted his local physician because of severe palpitations and had been given Pronestyl® 500 mg capsules three times daily. The palpitations promptly disappeared, but the patient had continued to take the medication. Procaine amide was not discontinued, but ventricular extrasystoles recurred in the absence of congestive heart failure or angina pectoris. Serum digoxin was not at toxic levels. The patient received an intravenous 500 bolus of lidocaine followed by 400 mg dilantin intramuscularly and was subsequently maintained on oral dilantin 100 mg three times daily. The arrhythmia disappeared and the various myasthenic symptoms began to subside 48 hours after cessation of procaine amide. By day 10 the myasthenia had returned to its original quiescent stage and the patient was able to resume full activities. The dilantin was gradually tapered and then withdrawn. The patient has been free of cardiac symptoms for the past 20 months and the myasthenia gravis has presented no further problems.

Discussion

It is well known that certain antibiotics, muscle relaxants, anesthetic agents, quinine and its derivatives, and procaine amide interfere with neuromuscular conductions and should therefore be avoided or closely supervised in patients with myasthenia gravis (2). However, we are aware of only one reported instance of subclinical myasthenia gravis having been unmasked by accidental exposure to one such agent, methoxyflurane (3). Case No. 1 presented here represents a similar unmasking of myasthenia gravis following the intake of quinidine and procaine amide and represents the first recorded case report of this type. While clinicians are well aware of such toxic side-effects as fever, rashes, gastrointestinal disturbances, arthralgias, hematologic disorders and individual idiosyncratic symptoms, neuromuscular weakness or paralysis is not observed unless the patient has an underlying defect at the neuromuscular junction.

The deleterious effect of quinidine in myasthenic patients was first noted by Weisman (4). Subsequently quinine was also used as a provocative diagnostic test for myasthenia gravis, but this was abandoned because of its inherent danger and cumbersomeness and was superseded by more accurate and less toxic tests. Quinine (tonic) water consumed in relatively small amounts has been known to exacerbate myasthenic symptoms (1). Patient WB did not begin to experience diplopia until after ingestion of 2.4 gm quinidine over a 72-hour period. This suggests that the patient's inherent acetylcholine deficit was minimal and that relatively high blood quinidine levels were required to produce even this minimal clinical weakness. The injection of 1 and 3 mg edrophonium intravenously produced no improvement while 10 mg after an appropriate interval caused incomplete improvement of left lateral rectus weakness. This is in partial agreement with experimental evidence which shows that edrophonium in small doses is without effect in neuromuscular blockade induced by intraarterial quinidine (6). As a matter of fact, if edrophonium is administered during the recovery phase, it can even produce an additional depressant effect, while calcium is an inconsistent quinidine antagonist (6). This finding, along with those reported by Kornfeld et al (7), suggests that calcium can enhance neuromuscular transmission in myasthenia gravis. Therefore, if a negative edrophonium test is obtained, an intravenous bolus of 0.5 gm calcium gluconate should be injected rapidly and its effects on muscle strength observed. While calcium may be effective for testing purposes, its therapeutic use in myasthenia gravis has been nil (7). Quinidine in small doses appears to act on the presynaptic area impairing either formation or release of acetylcholine (6). In larger doses quinidine exerts a weak curare-like action of the postjunctional depolarizing type (6). The evidence for direct muscle membrane depression of quinidine remains controversial (6, 8, 9). It would seem that the degree or timing of neuromuscular depression by quinidine is directly proportional to the amount of quinidine used and the severity of the myasthenic defect.

Procaine amide differs from procaine in that an amide group is substituted for an ester linkage, thus decreasing its rate of hydrolysis and allowing for higher blood levels (10). Its interference with neuromuscular conduction has been well documented (11–16). Procaine amide acts pre- and postjunctionally, but its presynaptic depression of acetylcholine formation and/or release is of greater importance (16). At low rates of nerve stimulation procaine amide exerts a curare-like effect on the postsynapse by reducing the sensitivity to acetylcholine, while during high frequency nerve stimulation or in low concentration procaine amide decreases the production and/or release of acetylcholine and causes significant reduction in the amplitude of nerve impulses in the nerve terminals (12, 15, 16). It has also been suggested that procaine may combine with the receptor-mediator complex which is relatively stable and that this triple component complex makes the muscle membrane less susceptible to propagation of impulses and contraction (12).

In each of the cases reported it required several days of drug therapy before onset of symptoms and additional time for peak effects to occur. It is known that maximal blood procaine amide levels are reached within one hour of oral intake, but the required tissue levels are probably not attained until much later (10, 11). The clinical sequence of events was reversed after administration of procaine amide was stopped. The time required to attain full recovery after cessation of the drug was considerably longer than that reported by Drachman (15). This may be explained by individual variation in the pattern of drug metabolism excretion and/or severity of the actual acetylcholine deficit.

As in quinidine-induced blockage, edrophonium tests provided no clear cut improvement unless at least 10 mg was used. Although experimental animal work was shown that the decreased response to tetanic stimulation after procaine is not reversible with neostigmine (17, 18), pyridostigmine was partially effective in one of our patients.

Both quinidine and procaine amide affect bulbar and peripheral muscles without preference, but apparently act only on affected myasthenic muscle groups. In neither patient did these drugs induce weaknesses that had not been observed previously. Although warnings have appeared in the literature against the possible hazards of diphenylhydantoin in myasthenia gravis (19), we have observed no such untoward side-effects. Similarly, propanolol and digitalis do not exert any depressing effects on neuromuscular conduction. The rapid intravenous administration of a 50 mg lidocaine bolus or a slow lidocaine drip over a six- to eight-hour period has not resulted in any aggravation of myasthenia gravis in two elderly cardiac patients. The intravenous injection of lidocaine does not produce decreases in the twitch response, although it can potentiate a succinylcholine- or d-tubocurarine-induced neuromuscular blockade. Large doses of intravenous lidocaine can suppress respiration, but this action is via the central nervous system and is not due to depression of neuromuscular conduction (20). However, in life-threatening situations where larger doses of lidocaine may be required, the attending physician should be prepared to insert an airway and have a mechanical respirator on standby. It should be remembered that anoxia or concomitant anesthesia can lower the threshold levels of a potential neuromuscular depressant.

Summary

Two cases are presented where quinidine and procaine amide unmasked previously unsuspected myasthenia gravis and aggravated a previously mild, stable case of clinical myasthenia gravis. The use of quinidine and procaine amide should be avoided in myasthenia gravis and the use of other antiarrhythmic agents is suggested. The clinical appearance of neuromuscular weakness during the administration of either of these two drugs should alert the clinician to the possibility of the presence of subclinical myasthenia gravis. A negative edrophonium test alone is unreliable under such circumstances and should be accompanied by an intravenous calcium test.

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Infection and Cell Metabolism*

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Man is numerically more microbial than mammalian, that is to say, human cells are a distinct minority of the total number of cells composing the complex aggregate we know as man. The interrelations between these microbes and man are incredibly varied. In some instances early in the evolution of life microorganisms invaded primitive procaryotic cells to form stable, durable and autonomous relationships. The mitochondrion of the cytoplasm represents the development of such an early infectious process which has an enormous impact on cellular metabolism. Originally a bacterium, the mitochondrion is the historical remnant of an infection of more complex cells. This relationship is now essential to both the cell and the organelle, is not communicable in the ordinary sense of the term and can hardly be spoken of as an infection any longer.

At the other end of the spectrum, acute infectious diseases also exert major effects on cellular metabolism. Some of these effects are the primary cause of disease symptoms in the host and, as such, are more or less characteristic of the causative infectious agent. Other alterations in cell metabolism occur as a consequence of normal responses to infection and are thus more or less consistent, predictable, and stereotyped for all agents. These alterations may have important nutritional consequences for the host, particularly in patients already suboptimally nourished. Much of our contemporary understanding of these processes are the result of the careful, systematic investigations of Beisel and his colleagues (1–3) and this discussion will draw heavily on their original observations.

The general nature of metabolic responses to infection are shown in Table I (1). They begin promptly after the infection has begun, usually long before clinical disease results. Some processes are set into motion by fever, and others may not be detectable until convalescence. These effects may be due directly to the microorganism or its toxins, or secondarily due to inflammation, fever, or hormonal or nutritional manifestations of infection. The profundity of the change can often be related to the severity and duration of illness, to effects of therapy, and to the genetic, immune, and nutritional makeup of the host. In addition multiple systems are invariably involved. They may simultaneously act upon diverse and interrelated metabolic processes. These processes may

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change individually or at different times during illness. Therefore, observations must be carried out in serial studies; simple, facile, mechanistic explanations for isolated observations at any one time cannot describe the entire complex of response to live microorganisms.

One way to classify the metabolic responses to infection is to divide them into anabolic and catabolic events. During febrile illnesses the catabolic events are most prominent and it is easy to overlook the simultaneous occurrence of important anabolic processes (1). Table II lists the diverse nature of these events, and the intimate involvement of many of these with host defenses. Production of large numbers of rapidly turning over phagocytic cells is often not considered in terms of the expenditure of energy and synthesis of protein necessary. However, marrow production of cells may be stepped up 5- to 10-fold. These cells, of course, are specialized to engage in phagocytosis of invading bacterial pathogens, during which cellular metabolism is sharply altered (4). For example, glycolysis is increased to fuel phagocytosis, lipid turnover is enhanced in large part to regenerate membrane fragments internalized as the phagocytic vacuole and direct oxidative metabolism of glucose and oxygen consumption are markedly stimulated in the generation of intracellular bactericidal systems. In addition, during phagocytosis at least two endogenous leukocyte mediators are produced and released which have

TABLE I

Cell Metabolism in Infection

1. Prompt response to infection
    - Stereotyped
    - Predictable onset
    - Related to severity
2. Direct actions of microorganism or biologically active products (e.g., toxins)
3. Indirect actions related to inflammation, fever, hormones or cellular mediators
4. Involvement of multiple, interrelated variables

TABLE II

Anabolic Response to Infection

1. Variable
    a. Nature of infection
    b. Timing
    c. Magnitude
2. Cellular turnover
    a. Phagocytic cells
    b. Lymphocytes
    c. Tissue repair
3. Protein turnover
    a. Immunoglobulins
    b. Complement
    c. Interferon
    d. "Acute phase" glycoproteins
    e. Leukocyte mediators

profound effects on the metabolism of other cells. These mediators, leukocyte endogenous mediator (LEM) (5) and endogenous pyrogen (6), are hormone-like in their role, inasmuch as they are released by secreting cells, travel via the blood stream, and direct processes occurring at distant sites. Looking first at LEM, its major impact is on the hepatocyte and cells of the RE system. Here LEM stimulates the uptake of huge quantities of iron, zinc, and free amino acids which have been released largely from breakdown of muscle protein (1, 3, 5). Its effect is not limited only to these transport mechanisms, but also results in the stimulation of RNA synthesis, formation of polyribosomes, and synthesis of serum alpha glycoprotein acute-phase reactants and ceruloplasmin. This increased synthetic activity also involves inducible enzymes such as tyrosine transaminase and tryptophan oxygenase, as well as haptoglobin, alpha-1-antitrypsin, alpha-1-acid glycoprotein, fibrinogen (which results in elevation of the erythrocyte sedimentation rate), C-reactive protein, ceruloplasmin, and seromucoid. These products have no protective role for the host as far as is known.

Leukocyte endogenous matter is a heat-labile, trypsin- and pronase-sensitive protein of small molecular weight (10,000–30,000 daltons) which is not affected by lipase, RNA-ase, or alterations of pH (1). The initial transport of amino acids induced by LEM is accomplished without activation of the adenylcyclase/cyclic AMP system. Subsequent protein synthetic activity involves new RNA synthesis first and requires the presence of glucocorticosteroids. Regulatory mechanisms are somehow employed to slow albumin synthesis as amino acid precursors are shunted into production of these "acute phase" globulins.

Fever is the result of resetting a thermostatic control mechanism in the hypothalamus which regulates heat loss and heat production mechanisms (4). It is one of the cardinal manifestations of disease and, like the erythrocyte sedimentation rate, is a nonspecific finding which is often extremely useful in following the clinical progress of a patient. Fever production in infection begins with a stimulus to the leukocyte to produce a mediator protein called endogenous pyrogen. This protein, a small molecular-weight material which may be closely related to LEM, circulates in the blood stream to reach sensitive cells in the hypothalamus. The thermostatic arc involves neural signals to increase heat loss principally via evaporation (sweating) or radiation, or to increase heat production principally by muscular activity (shivering, that is, the clinical chill) which converts energy to heat rather than work. Like the acute-phase glycoproteins, fever plays a largely unexplained role in the response of the body to infection.

Table III shows the principle catabolic events observed during infection. The major stimulus for initiating catabolic events appears to be fever itself (1). Catabolism of protein, largely from muscle tissue, serves to redistribute amino acids to organs engaged in anabolic events, such as the liver and bone marrow. The site of principle catabolism has been estimated in man by determination of urinary 3-methylhistidine (3-MH), an analogue of histidine produced from

TABLE III

Catabolic Response to Infection

1. Induction by fever
2. Redistribution of muscle protein
    a. Protein synthesis in other tissues
    b. Gluconeogenesis
3. Negative metabolic balance
    a. Nitrogen
    b. Potassium, phosphorus, magnesium
    c. Trace elements (e.g., zinc)
4. "Catabolic tachyphylaxis"
    a. Chronic cachexia

the contractile proteins, actin and myosin (7). 3-MH is neither reutilized nor degraded to CO_2, and hence serves as a choice marker for muscle protein turnover. These studies show excellent correlation between 3-MH excretion and overall nitrogen balance. Amino acids, again largely derived from muscle, are also used for gluconeogenesis in the liver. The overall nitrogen balance is negative during the febrile period, and may require a prolonged period of convalescent repletion to eradicate the deficit. Because free amino acids are not stored in the body, negative nitrogen balance is the equivalent of an absolute loss of protein. This is heightened by the fact that during infection there is no compensatory decrease in urinary nitrogen excretion.

Losses of K, P, Mg, S, and Zn all parallel that of nitrogen (1, 3). With chronicity of the infectious process, the daily balances increasingly become less negative and ultimately a steady state is reached (1). The plateau of this "catabolic tachyphylaxis" clinically is chronic cachexia.

There has been recent interest in the metabolism of trace metals, including copper, cobalt, manganese, gallium, and chromium during acute infectious diseases (1, 3). Alterations of iron metabolism have been intensively studied particularly with relevance to the etiology of the "anemia of infection." In virtually all infectious processes the serum iron concentration is low. Prospective studies show that iron levels drop during the incubation period, before the development of fever. The decrease in serum iron is greater than can be explained by decreased transferrin levels. There is, in fact, an increase in the clearance of iron from plasma, first to hepatocytes and then to the reticuloendothelial system. The earliest redistribution of iron appears to be under the influence of LEM into liver cells where it combines with apoferritin to form ferritin, and then is stabilized as hemosiderin. A major departure from this general rule is the elevated level found in infectious hepatitis, but the reason for this is readily apparent. Because of the central role of the hepatocyte in iron uptake, the cell damage due to viral hepatitis causes release of ferritin and/or alters transport of iron across the cell membrane which results in an elevation of the serum iron level.

Depressed zinc levels are equally characteristic of infection and parallel the shifts occurring with iron, including the effect of LEM which increases the uptake of Zn into hepatocytes. In contrast, serum copper levels increase but only after initiation of disease. This is due to enhanced hepatocyte synthesis of the carrier protein, ceruloplasmin, which is one of the acute-phase glycoproteins.

Beisel has classified nutrient wastage during infection as absolute or functional (2). The former is quite obvious and is reflected in negative metabolic balance studies, the classic way to perform these evaluations. The latter concept is far more interesting, and more subtle (Table IV). Functional wastage is accomplished by three basic mechanisms: excessive utilization, sequestration in inaccessible body pools, and diversion from usual metabolic pathways. The first mechanism is exemplified by the increased expenditure of metabolic fuels to provide for energy requirements of fever. Depletion of glycogen stores, gluconeogenesis from amino acid precursors, and mobilization of depot fat may all be involved. It is a functional wastage since we do not know the physiologic purpose of the febrile response. The second mechanism is exemplified by the sequestration of iron in hepatocytes and the R/E system where it is unavailable for hemesynthesis. The third mechanism, diversion of nutrients, is typified by the uptake of amino acids by the liver and the synthesis of acute phase reactants with no known function in host defense.

After discussing all of these relatively nonspecific metabolic responses, it is probably critical to emphasize direct effects of microbes or microbial products on cell metabolism, since this, of course, does occur. An increasingly understood example is the effect of Vibrio cholerae on intestinal epithelial cells. There is no doubt now that the disease is caused by production of a large (82,000) molecular-weight protein toxin by the organism (8). This protein is bound first to the brush border of the intestinal epithelial cell, from which the toxin, a fragment, or a mediator signals the activation of adenyl cyclase activity producing increased intracellular levels of cyclic AMP. This in turn tells the cell to secrete isotonic water and electrolyte, which is, of course, the

TABLE IV

*Functional Nutrient Wastage in Infection**

Varies in pattern or severity with different types of infections or inflammatory processes

1. *Overutilization*
    - Increased expenditure of metabolic fuels
    - Depletion of glycogen stores
    - Diversion of amino acids for gluconeogenesis
    - Mobilization of depot fat
    - Increased liver synthesis of cholesterol and triglyceride
2. *Sequestration*
    - Uptake of iron and zinc into hepatocytes and R/E system
    - Other trace metals
3. *Diversion*
    - Uptake of plasma amino acids by liver
    - Hepatic synthesis of "acute phase reactant" glycoproteins
    - Hepatic synthesis of enzymes (tryptophan oxidase, tyrosine transaminase, α-glycerophosphate dehydrogenase)

* Adapted from Beisel, W. R., ref. 2.

principal clinical manifestation of cholera. Data from experimental cholera in animals suggests that glycolysis is the source of energy for this active secretory process (9). The result of this is a profound diarrhea in which a subject can lose 20 or more liters of fluid daily, and, of course, this rapidly results in shock. The loss of fixed base and potassium causes acidosis and severe hypokalemia. The latter can be the cause of electrocardiographic abnormalities and hypokalemic renal tubular necrosis. Thus a series of direct and indirect metabolic events occurs during infection with the cholera vibrio.

Summary

Infectious diseases result in profound changes in cell metabolism. Both anabolic and catabolic events occur under different regulatory mechanisms and at specific times during the course of infection. Alterations in distribution and metabolism of various nutrients also occur, regulated by a mediator protein derived from leukocytes. These are described under three categories of functional wastage of nutrients: overutilization, sequestration and diversion. Finally, specific effects on cell metabolism by microorganisms on their products are described using V. cholerae and its enterotoxin as an example.

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Effect of ABO Incompatibility on Mean Coulter S and Bilirubin Values of Cord Blood*

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Abstract

Coulter S hematologic data and AutoAnalyzer serum bilirubin levels on 3589 cord blood specimens were analyzed in regard to the effect of feto-maternal ABO types. Anemia, hyperbilirubinemia, and leukocytosis were associated with the non-0 babies of type 0 mothers. The effect of ABO incompatibility on mean hemoglobin and bilirubin levels was exactly the same as that noted 18 years earlier.

In 1953 and the first half of 1954, cord bloods of babies delivered at The Mount Sinai Hospital were studied for hemoglobin level, serum bilirubin concentration, reticulocyte count, and the result of a carefully interpreted manual direct IgG antiglobulin test. A report for the first 9 months of 1953 disclosed the predominance of type O mothers associated with ABO incompatible babies with positive antiglobulin test(3). These babies, as a group, also had lower mean levels of cord blood hemoglobin and higher mean levels both of bilirubin and reticulocytes (3). A second study, over an 18-month period which included the earlier one, disclosed similar findings(5). However, with larger numbers of cord bloods studied, ABO incompatible babies that were IgG antiglobulin test negative also showed significant abnormalities of the same hematologic parameters (5). In addition, an excess of type A babies over type B babies was observed among antiglobulin positive offspring of type O mothers (5).

In 1971 and 1972, another study was initiated to determine whether additional factors altered hematologic values of cord blood. For this, over 3,500 mother-baby pairs were studied and the data in reference to a much larger number of variables were cardpunched. Analysis of these data is still in progress, but an initial effort was made to determine whether cord blood hemoglobin and bilirubin values differed in any significant manner from those reported in 1953–4, and whether the ABO type of the baby or the mother had any hitherto unrecognized effects on hematologic values.

A comparison between these two studies had to be restricted to computation of mean values for hemoglobin and bilirubin; reticulocyte counts were no longer routinely performed. Furthermore, the direct manual IgG antiglobulin test in 1971 and 1972 was performed in routine rather than special fashion and no longer provided a large number of positive results; only 43 (1.2%) were

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noted. Accordingly, positive antiglobulin tests were not handled as a separate class. In both studies, a baby whose mother had unexpected serum hemagglutinins was excluded.

Methods

Cord blood specimens. In both studies, cord blood was obtained by needle and syringe from the umbilical vein attached to the placenta. This was done following the third stage of labor and after the cord had been severed. Each specimen was divided into two portions, one clotted and the other anticoagulated. In 1952–3, balanced oxalate (3, 5) was the anticoagulant; in 1971–2, E.D.T.A. was used (test tubes number 3204 QS, Becton-Dickinson Co., Rutherford, N.J.)

Determination of hematologic values. In 1953–4, both hemoglobin and bilirubin levels were determined manually (3, 5). In 1971–2, bilirubin was determined by AutoAnalyzer (4) while hemoglobin, erythrocyte, and leukocyte data were obtained by Coulter S (Coulter Electronics, Inc., Hialeah, Fla. 33014). Standards for both hemoglobinometry and bilirubin assay differed in the two study periods. The 1971–2 standard for hemoglobin (1) provided about 1.5% higher values than were observed in 1953–4. The bilirubin standard for bilirubin (4) in 1971–2 yielded over 10% higher estimations than were obtained in 1953–4 with bilirubin in chloroform (3, 5).

Results

For the 1971–2 study, all data were examined in reference to 8 classes of babies: (1) type O babies of type O mothers; (2) type A babies of type O mothers; (3) type B babies of type O mothers; (4) type A babies of type A or AB mothers; (5) type B babies of type B or AB mothers; (6) type O babies of type A or type B mothers; (7) type A or AB babies of type B mothers; and (8) type B or AB babies of type A mothers. Within each of these classes, the results found for each variable were assorted according to size into discrete, evenly spaced, quantitative categories to determine whether the results were distributed normally. Bilirubin data, and all Coulter S data except leukocyte counts were distributed normally, but all classes of babies showed many instances of high leukocyte counts and, accordingly, skewed distributions. This could be normalized by logarithmic transformation of leukocyte counts.

For each of the 8 classes of babies, mean and variance values were obtained for bilirubin and the primary measurements from Coulter S tests, namely hemoglobin concentration (Hgb), erythrocyte count (RBC), mean erythrocyte volume (MCV), and leukocyte count (WBC). Except for the known effects of materno-fetal ABO incompatibility, ABO types of babies were not associated by analysis of variance with significant differences in either bilirubin or the non-leukocyte primary data from Coulter S evaluations. Accordingly, these data were pooled into 3 classes: (1) all ABO compatible babies; (2) type A or B babies of type O mothers; and (3) ABO incompatible babies of non-O mothers. Logarithmically transformed leukocyte data were also pooled in this manner, despite an extraordinarily high observed variance for type B babies of type O mothers. The pooled data and results of statistical analysis are given in Table I.

Three other measurements are derived mathematically by the Coulter S instrument: hematocrit (Hct) = RBC × MCV; mean corpuscular hemoglobin (MCH) = Hgb ÷ RBC, and mean corpuscular hemoglobin content (MCHC) = Hgb ÷ (RBC × MCV). None of these measurements varied significantly between classes of babies except for the observation that the mean and variance values for Hct were higher for type A babies of type O mothers ($n = 380$, $\bar{X} = 48.84\%$, $s^2 = 37.20$) than for type B babies of type O mothers ($n = 156$, $\bar{X} = 47.30\%$, $s^2 = 26.25$). The difference in means was significant at $P = 0.003$ while the variance ratio indicated $P \sim 0.01$. The mean values and their standard deviations for all 3,589 babies studies were: Hct = 49.94% ± 5.39, MCH = 35.42 pg ± 1.99, and MCHC = 32.31% ± 1.48.

The ABO incompatible babies of non-O mothers were found to have significantly lower mean hemoglobin levels but did not differ significantly in RBC, MCV, WBC, or serum bilirubin concentration from ABO compatible babies. In contrast, type A and type B babies of type O mothers displayed highly significant reduction of both Hgb and RBC, and elevation of serum

TABLE I

Effect of ABO Incompatibility on Mean Hematologic Values of Cord Blood. Data from 1971–2

Measurement	ABO Compatible Number of Babies → 2829	ABO Incompatible	
		Mother O 536	Mother not O 224
Hemoglobin (g/100 ml blood)			
mean	16.3190	15.731*	16.016*
variance	2.7578	2.9761	3.1325
Erythrocytes ($\times 10^6/\mu$liter blood)			
mean	4.599	4.4152*	4.519
variance	0.4781	0.5260	0.4962
Mean cell volume (μm^3)			
mean	108.8756	109.2369	108.683
variance	39.2674	43.9793	39.9125
Leukocytes (ln‡ [WBC/μliter blood])			
mean	2.3834	2.4330**	2.3917
(Geometric mean)	(10,842)	(11,393)	(10,932)
variance	0.09895	0.12916†	0.10490
Bilirubin (mg/100 ml serum)			
mean	2.356	2.549**	2.313
variance	0.3941	0.6618†	0.4267

*t value for difference from mean value of compatible babies is significant at $P \leqslant 0.01$.

**same as * but variances are assumed to be heterogeneous.

†variance ratio is incompatible: compatible is significant at $P \leqslant 0.01$.

‡ln = natural logarithm.

bilirubin and WBC. MCV values did not differ significantly, but were slightly elevated for non-O babies of type O mothers.

For both bilirubin and leukocyte counts, the variance value for non-O babies of type O mothers was much greater than the variance value for compatible babies.

The mean hemoglobin and bilirubin values were calculated for all babies studied in 1953–4 (5) and in 1971–2. The ratio of these mean values for (1971–2)/(1953–4) was found to be 1.01513 for Hgb and 1.1062 for bilirubin.

The actual mean values and their variances for ABO compatible and the two kinds of ABO incompatible babies studied in 1953–4, are given in Table II. Assuming that the mean values of all babies studied in 1953–4 would be the same as those of babies studied in 1971–2 if both studies had employed the same reference standards, the mean values for the 1953–4 study were corrected by the (1971–2)/(1953–4) ratios of mean values, and these corrected mean values are also given in Table II. As expected of the 1953–4 data, mean Hgb and bilirubin values for ABO incompatible babies of type O mothers were distinctly different from those of ABO compatible babies. In contrast to the data of Table I, the mean Hgb of ABO incompatible babies of non-O mothers did not differ significantly from that of ABO compatible babies.

The mean values obtained in 1953–4, corrected by the (1971–2)/(1953–4) ratios were compared with the mean values of 1971–2. No significant difference in mean values was discernible by t-test. Variance values were also compared. For Hgb, F-ratios were not significantly different from unity but, for bilirubin, variance was 34% to 40% higher in 1953–4.

One other discrepancy was noted between the respective data for 1953–4 and 1971–2: the distribution of ABO types had changed. This is to be seen in

TABLE II

Effect of ABO Incompatibility in 1953–4 (5)

	ABO Compatible	ABO Incompatible	
		Mother O	Mother not O
Hemoglobin (g/100 ml)			
number	2256	416	229
variance	2.7255	3.1326	2.9803
mean	16.0497	15.5714*	15.9175
corrected° mean	16.2925	15.8070	16.1583
Bilirubin (mg/100 ml)			
number	2193	396	221
variance	0.5267	0.9266†	0.5938
mean	2.11081	2.3860**	2.1860
corrected° mean	2.3321	2.6395	2.4182

° corrected by the 1971–2/1953–4 ratios for all specimens, 1.01513 for hemoglobin and 1.10624 for bilirubin.

* t value for difference from mean of compatible babies is significant at $P \leqslant 0.01$.

** same as * but variances are assumed to be heterogeneous.

† variance ratio of incompatible: compatible is significant at $P \leqslant 0.01$.

Tables I and II for number of babies in each of the three classes. There was an increase in type O from 41.54% (± 0.915) to 47.04% (± 1.145).

Discussion

This study provides statistical data in regard to serum bilirubin levels, Coulter S blood count information, and the effect of materno-fetal ABO incompatibility on each of these measurements. ABO incompatibility associated with type O mothers was associated with a highly significant decrease in both hemoglobin and red cell count mean values, and an increase in serum bilirubin. It was also associated with significant leukocytosis. MCV, however, was not significantly changed, although a slight observed increase seen with ABO incompatibility could have reflected increased reticulocytosis.

Derived mean Coulter S values, MCH and MCHC, failed to provide information relative to ABO incompatibility, but Hct data indicated that the mean value for type B babies of type O mothers was lower, and its variance was higher, than values for type A babies of type O mothers.

Variance values were least with ABO compatibility and almost always greatest with non-O babies of type O mothers. This could have resulted from active ABO disease cases within the ABO incompatible classes. Indeed, "disease" could not only broaden the distribution curve but cause skewness as well. A clearly significant variance ratio was seen with bilirubin, both in 1953–4 (Table II) and 1971–2 (Table I). Bilirubinemia in cord blood is highly associated with ABO hemolytic disease. In contrast, fetal bleeding, like ABO hemolytic disease, can also lower cord blood hemoglobin concentration and red cell count. Variance ratios for incompatible:compatible babies were not significant in 1971–2 (Table I), and only in 1953–4 was the hemoglobin variance ratio high (Table II).

All classes of babies were associated with skewed distribution of leukocyte counts. When leukocyte counts were handled as logarithmic values, normal distributions were obtained. With this transformation, variance values were also uniform except for type B babies of type O mothers. This variance value, however, was so high that a pooled variance for type A and type B babies of O mothers was very much greater than the variance of compatible babies. Both type A and type B babies of O mothers displayed highly significant elevations of their mean leukocyte counts. For B babies, this was also associated with a significant increase in variance.

The results of the 1971–2 study were compared with two variables, hemoglobin and bilirubin, studied in 1953–4 (3, 5). Methods and standards had changed somewhat, and mean values for 1953–4 were corrected to correspond to those of 1971–2 by use of a ratio derived from the mean of all babies in each study period. This adjustment may not be entirely valid. For example, nutrition of the mother may have an effect on cord blood hematologic values and maternal nutritional status may not have been the same in both study periods. Nevertheless, without such an adjustment comparison would not have been possible because standards for both hemoglobinometry (1) and bilirubin assay (4) had changed. With the adjustment, comparison disclosed no significant differences in the mean values obtained in studies separated by 18 years. This implies a consistent biologic effect of ABO incompatibility.

Variance values for hemoglobin differed very little in the two study periods, suggesting that manual hemoglobinometry in 1953–4 was not less accurate than automatic Coulter S data in 1971–2. In both studies variability appears to arise largely from individual differences. This was not the case for bilirubin where variance in 1953–4 was 33–40% greater than in 1971–2. Here, however, instrumentation by AutoAnalyzer had been adopted specifically to achieve greater reproducibility (4). Despite the increased variance observed for bilirubin in 1953–4, variance in that study period for O mother-not O baby was much more than for ABO compatible babies ($P < 0.01$), just as was observed in 1971–2.

A significant difference in the frequency of ABO types was observed between the two study period ($P = 0.02$). Unfortunately, there is no longer any available record of obstetric ABO types in 1953–4, but it is to be noted that legalized abortion began in 1971. This unexpected change in distribution of ABO types highlights the consistency of the effects of ABO incompatibility, which were identical not only over an 18-year period but despite a major change in ABO blood type frequencies.

The hematologic values herein reported differ slightly from other published data (2). This could reflect differences in reference standards, differences between tested populations, average gestational age of delivered babies, or quality of cord blood specimens in reference to changes in vitro between times of collection and actual assay and in reference to placental umbilicus versus venous blood from the baby. Placental blood is not likely to be associated with rapid redistribution of water (6) and, except in active hemolytic disease, displays, in our experience, negligible sedimentation of erythrocytes. Placental blood, therefore, should be preferable material for analysis.

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Serous Otitis Media and Mastoiditis: Their Radiologic and Clinical Manifestations

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The entity of serous otitis media (SOM) has been recognized clinically by otolaryngologists and pediatricians for many years (1, 2). Its radiologic manifestations have not been appreciated by most radiologists for a variety of reasons. First, this entity has not been described in the radiologic literature, and second many of the cases referred to the radiologists with SOM have either been mistaken for suppurative mastoiditis, or else have been thought to be within normal limits with asymmetry of mastoid development (3).

Definition: SOM is an inflammation of the middle ear and mastoid, occurring mainly in children, caused by a malfunction of the eustachian tubes. There is retention either of mucoid or purulent secretions within the middle ear, often associated with a hearing deficit (4). Adenoidal inflammation in children and upper respiratory infection, sinusitis, allergy, or aerotitis in adults are the most common causes of abnormal function of the eustachian tubes (5).

Material: Over 1,000 cases of SOM have been surgically treated (S.S.F.) in the past ten years. Approximately one quarter of these have had preoperative x-rays of the mastoids and temporal bones (C.B.). Postoperative follow-up x-rays were performed in about 10% of these cases, ranging between six weeks and one year after the drainage procedure (Cases 1, 5, 6, 8). More than 90% of the patients were children six weeks to twelve years of age; the remainder were adults.

Discussion

This study was undertaken to elucidate the roentgen findings in serous otitis media and mastoiditis and to correlate the radiographic abnormalities with the clinical manifestations, degree of severity, and extent of the disease in each ear. It was also hoped that by accumulating a large experience with careful clinical follow-up the radiologist could help the clinician in planning the treatment and formulating the patient's prognosis.

Conventional therapy of SOM with antibiotics and antihistamines has usually failed. Therefore, we have performed frequent radiologic examinations of the mastoids in an attempt accurately to diagnose the extent of mastoid involvement in those resistant cases.

The routine radiographic positions used for this study were: Towne's, Schuller, Mayer's, and Stenver's projections. In infants, sedation was usually

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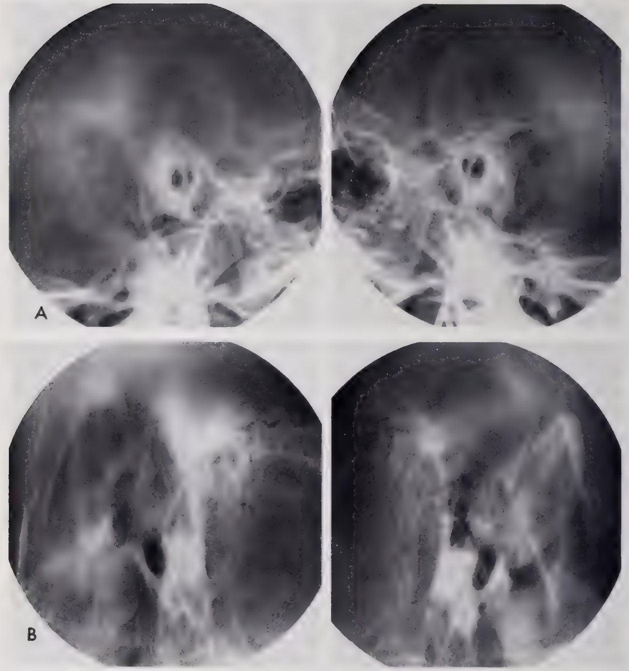


CASE 1

FIG. 1 A. Law's projection of both mastoids reveals a well-developed and moderately cloudy right mastoid with intact septa. On the left, the cells are less well developed, with periantral clouding.

FIG. 1 B. In the Mayer's view, the cloudy right mastoid is well seen with distinct and intact septa. On the left, the septa are thickened, but the cells are not cloudy.

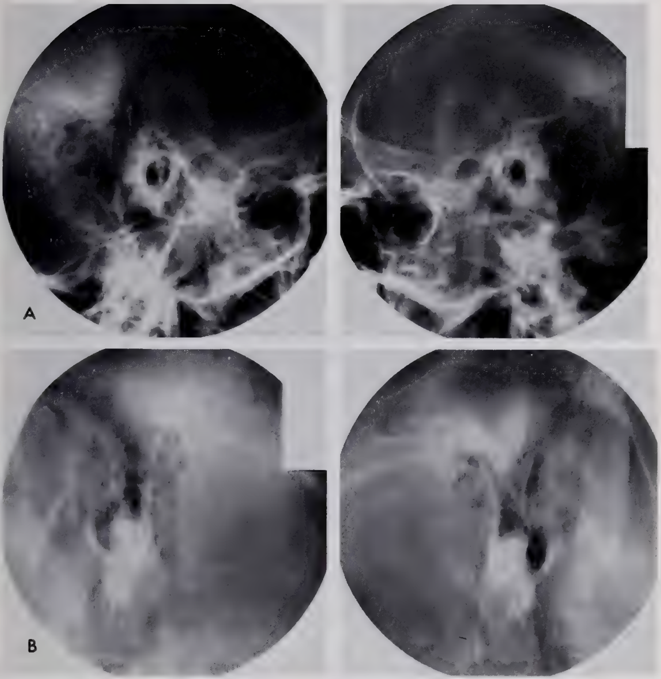
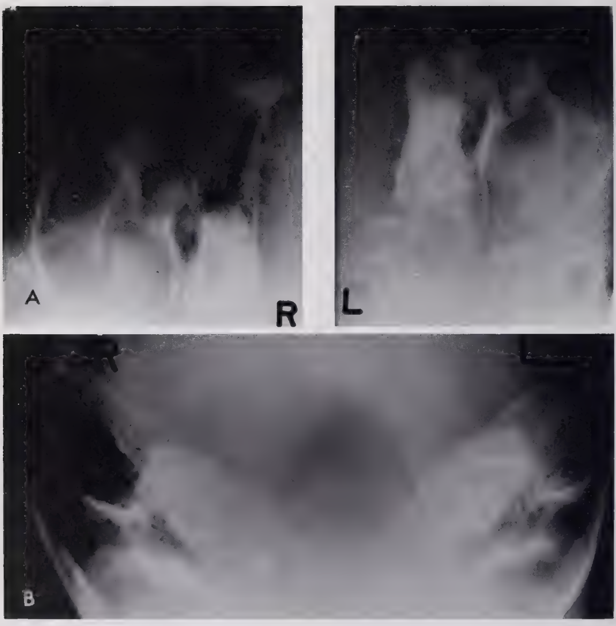


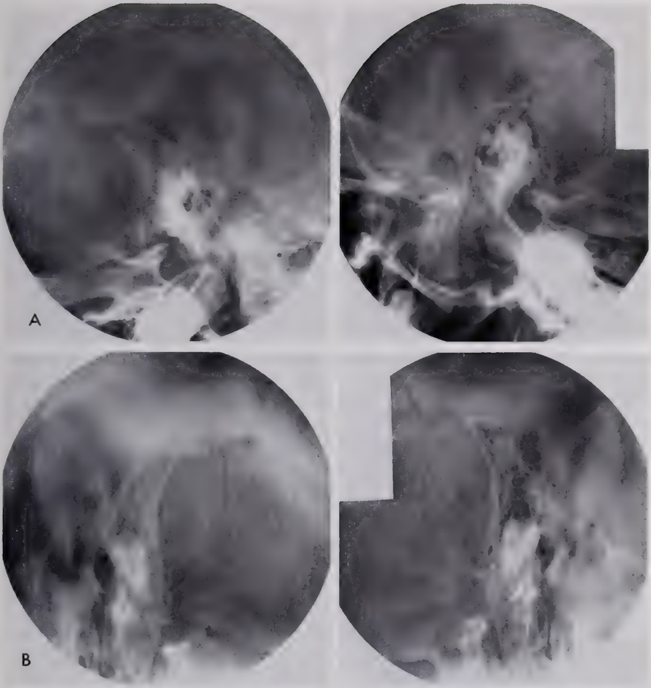
FIG. 2A&B. After adenoidectomy and bilateral middle ear drainage, the right mastoid cells appear clear and well pneumatized; on the left, the septa are slightly thickened, but the cells are clear, with minimal residual periantral clouding. This postoperative change suggests an acute SOM on the right and a mild subacute condition on the left.



CASE 2

FIG. 1A. On the Mayer's projection, the right mastoid is well developed and normal. On the left, there is marked clouding of a well-developed mastoid. The septa are sharp.

FIG. 1 B. In the Towne's view, the unilaterality of the disease is well demonstrated. This is a striking feature in this case of acute SOM, where the mastoid development was complete prior to the onset of the disease.



CASE 3

Fig. 1A&B. The right mastoid cells are less well developed than those on the left. There is clouding of the posterior and superior periantral cells. On the left, the mastoid is normal. This six-year-old patient had symptoms for one year and was thought to have subacute SOM on the right.

required to obtain diagnostic films. Tomographic sections of the mastoids were done in a number of suitable cases, but revealed no additional information in SOM.

Clinical Manifestations: The patients usually had several episodes of otitis media following upper respiratory infections in childhood, often treated with repeated courses of antibiotics. When SOM was present, they exhibited conduction deafness; and pneumatic otoscopy often revealed fluid in the middle ear (6). Retraction of the tympanic membrane with or without granuloma was usually present in chronic cases.

Tonsillectomy and thorough adenoidectomy to improve eustachian tube drainage was the operation of choice. The question of whether an aerating teflon drainage tube should be inserted after myringotomy was helped by prior knowledge of the extent of mastoid involvement on x-ray. It has also been our feeling that the teflon tube should be left in situ when the aspirated material from the middle ear was glue-like in consistency (7).

Roentgen Findings: The radiologic findings is SOM and mastoiditis vary according to the age at the onset of the disease. SOM can be subdivided into acute, subacute, and chronic varieties.

In the acute phase of the disease, when the mastoid cells are already developed, the most common finding is clouding of the cells starting in the periantral region. The intercellular septa become indistinct, but no case of intercellular bone destruction has been found in our entire series (Cases 1 & 2). When proper treatment is instituted and the patient is restudied, the mastoids revert to normal (Case 1). In the acute cases, there is no retraction of the sinus plate or depression of the tegmen tympani.

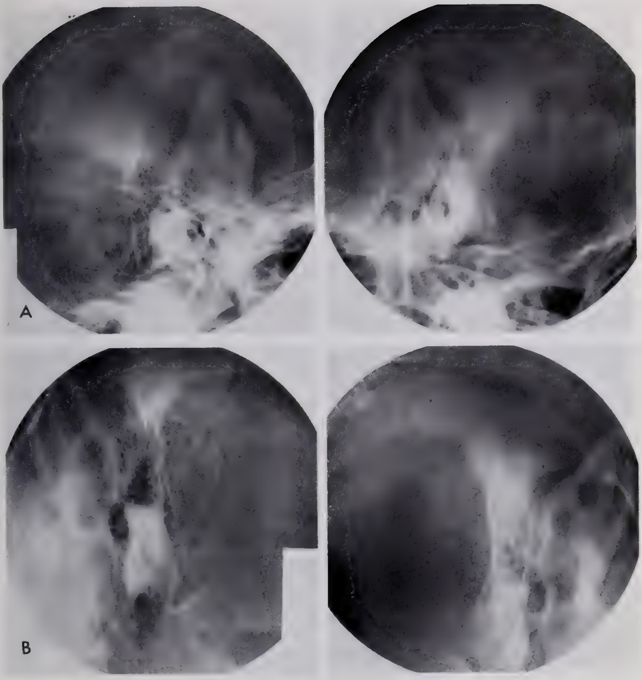
When SOM becomes subacute, there is often asymmetry of mastoid development, with poorer pneumatization on the affected side. This is most striking in patients over the age of five years with unilateral disease (Cases 3, 4). Most of these patients have a moderately long history of recurrent otitis media, often dating back to early childhood; one would therefore expect underdevelopment of the mastoid air cells—theory of Wittmaack (8–10).

In the chronic cases, the mastoids may be sclerotic or markedly underdeveloped. There is frequent anterior displacement of the sinus plate and depression of the tegmen tympani (Cases 9, 10). This is the result of previous softening of the mastoid bone and constant pulsation and pressure of the overlying dura.

Frequently, when the disease was thought to affect only one ear by history and clinical examination, the radiographic findings point to bilateral disease (Cases 7, 8). There have been no cases of septal breakdown, bony destruction, or cholesteotoma formation in our series.

Radiographic studies following proper drainage procedure have shown that in acute cases the mastoids can revert to a normal appearance (Case 1). In the subacute cases, the affected mastoid may shown marked improvement in the degree of cellular aeration and revert to a more normal state (Cases 5, 6, 8).

The only common disease which has to be differentiated from SOM is



CASE 4

FIG. 1A&B. On the right, the mastoid cells are well pneumatized and normal. On the left, there is marked clouding of the cells throughout the mastoid region with intact septa. The degree of mastoid development is much less on the left than on the right, suggesting a subacute duration.

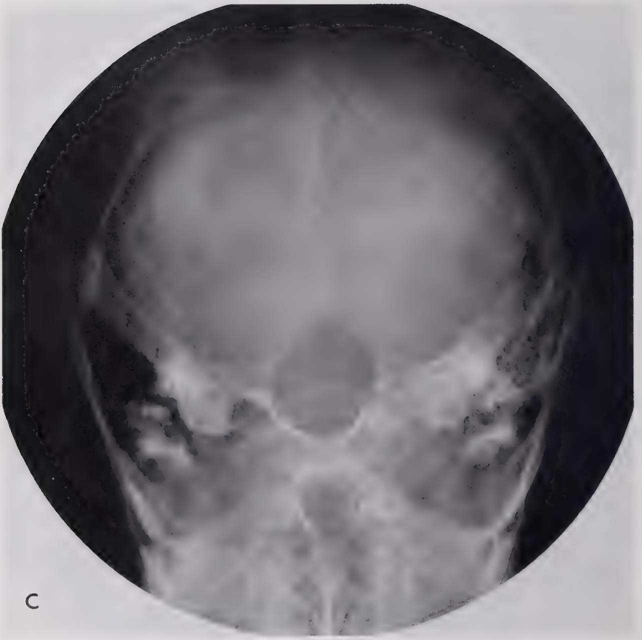
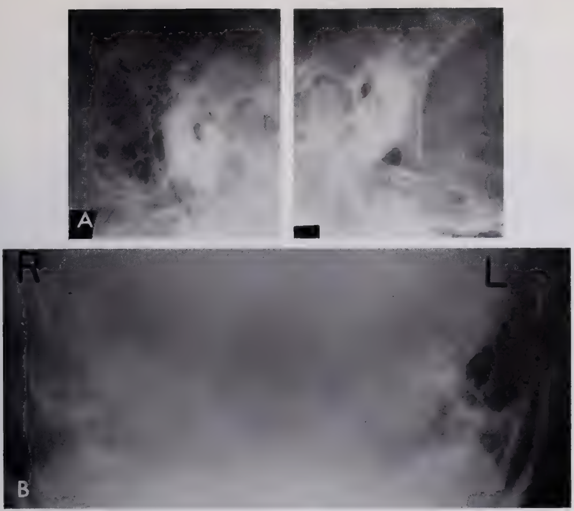


Fig. 1 C. The unilateral nature of this case is well demonstrated in the Towne's projection.



CASE 5

Fig. 1A&B. In this unilateral case, the right mastoid is normal and the left is markedly cloudy and poorly developed. The septa are thick but intact. This ten-year-old patient had a one-year history of unilateral disease and underwent adenoidectomy, myringotomy, and teflon tube insertion.

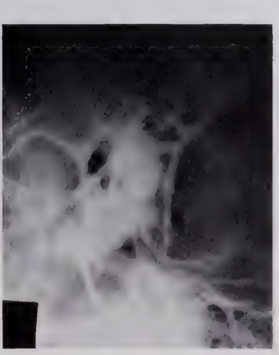
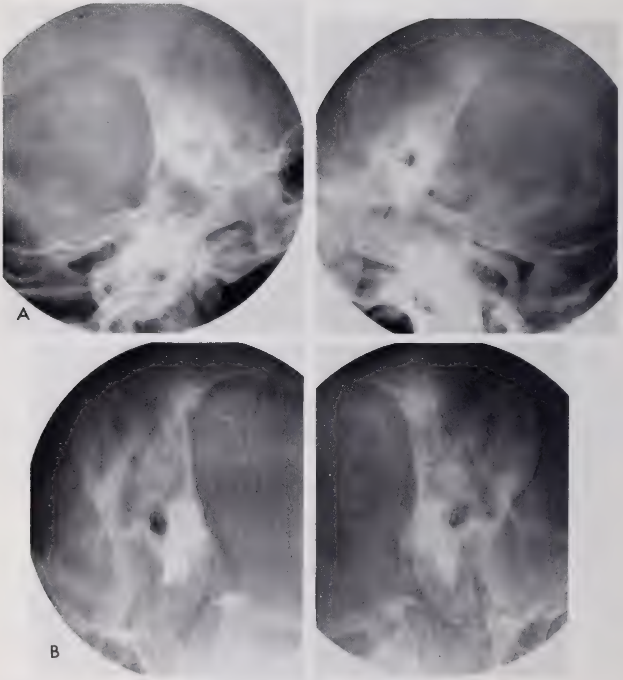


Fig. 2. Four months after surgery, the left mastoid is now seen to have cleared considerably with residual thickening of the septa.



CASE 6

Fig. 1A&B. This six-year-old patient with bilateral subacute SOM had marked clouding of the cells with intact septa and moderately well-developed mastoids.

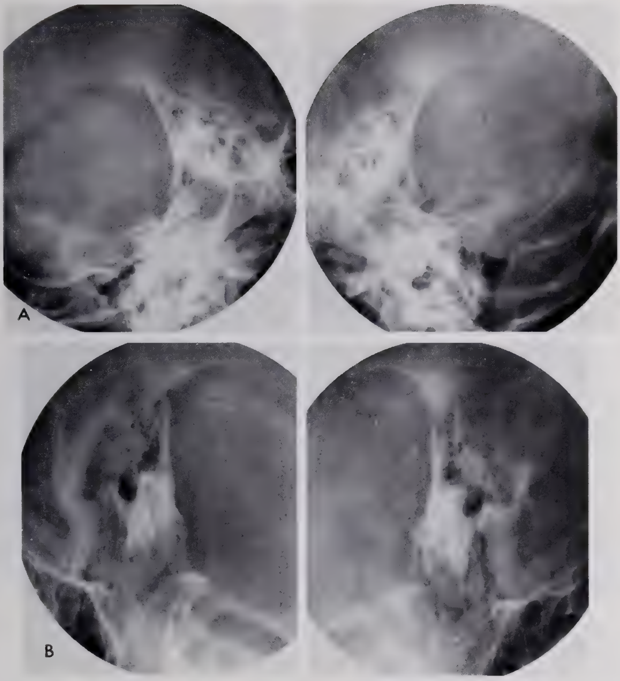
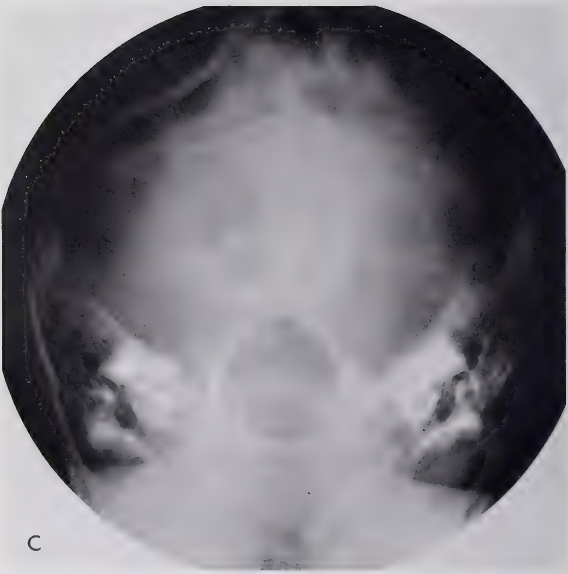
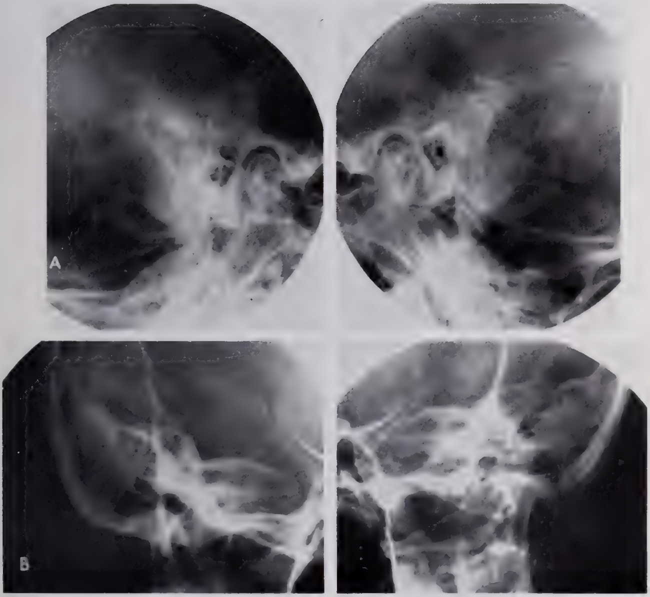


FIG. 2AB&C. Six months after surgery, the patient showed marked improvement, with clearing of the cells bilaterally.



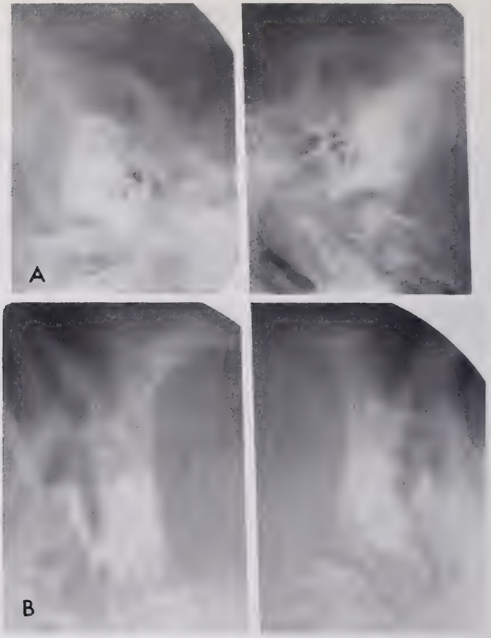
Case 6.
FIG 2C.



CASE 7

FIG. 1 A. In this patient, there is definite evidence of bilateral moderately advanced subacute SOM. There is much cellular clouding and septal thickening. The mastoids are diplopneumatic in type. Clinically, the disease was thought to affect the left side only.

FIG. 1 B. In the Stenver's view, the diplopneumatic architecture of the mastoid is well seen, with bilateral cloudy cells.



CASE 8

FIG. 1A&B. In this three-year-old patient, there is evidence of chronic SOM on the right, with poor development, marked clouding, and some retraction of the walls. On the left, the disease appears to be more subacute in type.

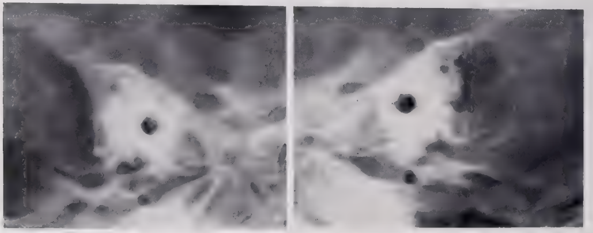
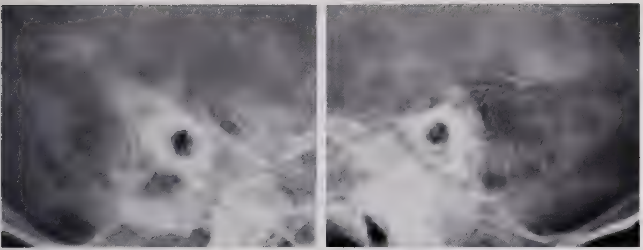
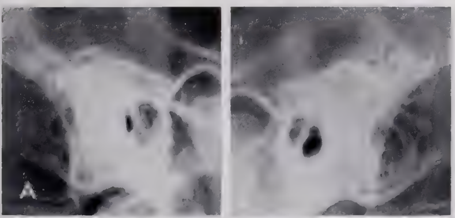


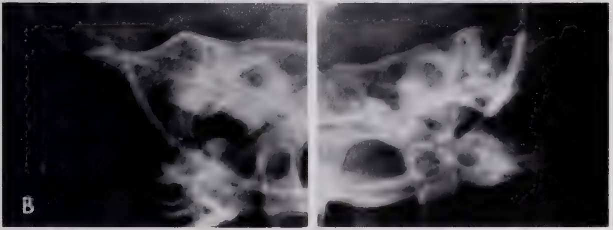
FIG. 2. Seven months after surgery, good aeration has returned to both sides and the right mastoid has begun to develop more normally.



CASE 9

Fig. 1. The retraction of the sinus plate is readily appreciated in the Law's view in this case of unilateral chronic SOM.





CASE 10

Fig. 1 A. There is definite retraction of the sinus plate on the right side as seen in the Law's projection. The mastoid is sclerotic and very cloudy. On the left side, the mastoid is also cloudy, but there is no retraction.

Fig. 1 B. In the Stenver's view, the depression of the posterior portion of the tegmen tympani is apparent on the right side as compared with the left.

purulent mastoiditis. In the latter condition, unilaterality is more frequent and there is often evidence of bone resorption within the septa and bony walls. None of our cases of SOM and mastoiditis have demonstrated radiographic evidence of bony destruction; cholesteotoma formation rules out the diagnosis of SOM and mastoiditis.

Summary

Over 1,000 cases of SOM and mastoiditis have been surgically treated by adenoidectomy and middle ear drainage. One quarter of these patients had preoperative mastoid x-rays, and 10% had postoperative follow-up studies.

This work was done accurately to describe the radiologic findings and to help in the treatment planning and prognostication of this common entity, which has never yet been described in the radiologic literature.

Cases can be divided radiologically into acute, subacute, and chronic, and vary in appearance according to the age at the onset of the disease. In acute cases, only cellular clouding is noted, which is reversible after therapy. In subacute cases, there is asymmetry of mastoid development, with poorer pneumatization on the affected side. In the chronic cases, the mastoid is sclerotic with retraction of the sinus plate and depression of the tegmen tympani. Frequently, when the disease is clinically thought to only involve one ear, bilaterality is detected radiologically. Septal or bony destruction rules out the diagnosis of SOM and mastoiditis and points instead to purulent mastoiditis.

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Evaluation of the Use of Syllabi in Teaching

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A syllabus is defined in Webster's International Dictionary as "a compendium or summary outline of a discourse, treatise, course of study, or examination requirements." Syllabus has been a word of infrequent use in educational circles. A more common reference is the word "hand-outs." Many educators use hand-outs quite unconsciously within the framework of the term syllabus. That is, a hand-out is commonly used to provide supplementary information not to be found in a pertinently organized fashion in a textbook or in the literature or of more recent nature. The hand-out or syllabus is designed to guide a student toward the study goals considered by the instructor to be more pertinent to his immediate objectives than are currently available in texts or references.

As study areas become more complex or more interdisciplinary, the need for study guides or syllabi increases. The author of a text selects from among a number of approaches to a subject the one which seems to him to be the most preferable. The instructor of a group of students ideally peruses these texts and the literature to develop a sum total of information gleaned from a number of sources. Thus, a single text or a group of references may not be adequate as study guides. The student might well benefit from a study guide prepared by a particular instructor for his own individualized course.

The founders of integrated medical teaching recognized early that the integration of concepts from anatomy, histology, embryology, biochemistry, physiology, pharmacology, microbiology, and pathology would not be possible unless the students had a study guide or syllabus to help them through the maze of textbooks full of information in these subject areas. This problem is not as likely to exist when the lecture material is discipline-oriented. For instance, the biochemist can lecture from a single recommended text of which there happens to be a variety. The anatomy lecturer also has available several excellent texts.

A student in an integrated curriculum could find himself turning from one textbook to another in an effort to bring the multiple disciplines to bear on a given subject area. The syllabus could be the bridge between a given organ system, cardiovascular, renal, respiratory, neurology, reproduction, and

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others, and the many discipline-oriented sources of information about these systems.

Medical school faculties in discipline-oriented departments have tended to resist interdisciplinary teaching. Each discipline seems more comfortable with the ease of teaching in a discipline isolation. Correlation of information from different disciplines on a common concept or a single organ system becomes the province of the student. This correlation requires intense application and considerable time, in inverse proportion to the knowledgability in the area. Thus, the medical student novice can spend hours gleaning the information from a variety of texts. On the other hand, the knowledgable instructor can visualize the information sources in seconds. With a little more effort the instructor can provide invaluable assistance to the student and guide him along a more efficient path of learning.

This study was designed to explore student opinions on the value of syllabi in medical teaching, and to determine, if possible, which aspects of syllabus use are most important to students. This initial study was directed to use of syllabi in general, rather than to the evaluation of a particular syllabus. It was hypothesized that students would evaluate syllabi quite favorably. It is hoped that results of this nature will encourage instructors in the development and/or improvement of syllabi for their courses.

Method

Subjects: The subjects for this study were 78 (out of approximately 150) medical students, at Mount Sinai School of Medicine, who voluntarily completed and returned a rating form sent to them by mail. The sample included 27 first-year students, 15 second-year students, 21 third-year students, and 15 fourth-year students.

Instrument: A rating form consisting of ten items was developed to obtain student judgments regarding the use of syllabi (Fig. 1). All items relate directly to the evaluation of syllabi in general, rather than to the syllabus prepared for any one course. All items selected were considered appropriate by three judges very familiar with the use of syllabi in teaching and the problems involved with this instructional tool.

Each item was followed by a seven-step scale with the terminal points labeled and each step numbered, as shown in Figure 1. Half of the items were designed to be favorable to syllabi and half were unfavorable to minimize possible response sets of subjects. Students also were asked to include on the form any additional comments they might have regarding syllabi. Instructions to the students for completing the rating form included the definition of each of the seven steps on the rating scale.

Reliability of the rating form was determined by the split-half method using odd versus even items. The correlation between halves was substituted in the Spearman-Brown formula for double length resulting in a correlation coefficient of .76. Test-retest reliability would have been preferable, but was not practical in an attempt to get wide student participation on a voluntary basis.

Rating Form

Check one: __ First Year, __ Second Year, __ Third Year, __ Fourth Year

Remember that the following statements refer to syllabi in general, and not to the use of syllabi in any particular course. Please check only *one* space for each item.

1. Are useful study guides for interdisciplinary teaching.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
2 Show conscientious or directed faculty effort to assure that students get information that is needed.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
3. Are an unnecessary duplication of material in textbooks and other reference sources.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
4. Foster dependency upon instructors to organize and provide study materials.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
5. Eliminate or limit the need to take extensive lecture notes.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
6. Is a form of "spoon-feeding" of students.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
7. Eliminate the need to attend lectures.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
8. Eliminate the need to utilize other sources of course-related information.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
9. Assist students in recognizing important concepts and facts.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree
10. Provide more up-to-date information than textbooks.	Completely disagree	__ : __ : __ : __ : __ : __ : __ 1 2 3 4 5 6 7	Completely agree

Any additional comments:

FIG. 1. Form used for syllabus survey.

Results

Scores were based on integral weights, from one to seven. Weighting of items was reversed for negative, or unfavorable, items. Mean scores were determined for each class and for the total group of students for both individual items and the total test. The mean scores for items and for the total test are presented in Table I.

The class that rated the use of syllabi highest was the first-year class, with a total mean score of 6.1 (out of a possible 7), with the third-year next with a rating of 5.6, and the second- and fourth-year nearly the same with ratings of 5.1 and 5.0 respectively.

The data show that all classes rated the use of syllabi quite highly. The greatest difference among the classes was between the first-year students and the fourth year students, a difference of 1.1 steps on the rating scale.

Approximately thirty percent of the students made additional comments on the rating form, and a large majority of these comments were favorable toward syllabi. The point emphasized by a number of these students was that the value of a syllabus is directly related to its quality, which depends upon the time and effort put forth by an instructor in its preparation.

Discussion

This study has tested the value of an educational tool for four levels of students involved at the time of the study in a complex educative process, developed in a structured curriculum by ten primary disciplines and twelve dependent secondary disciplines.

The students in this study reflected the spectrum of the undergraduate medical educational process. The first-year students had one year of experience with a variety of syllabi of varying quality. The variation in quality provided a basis for subjective comparison. The second-year student had two

TABLE I

Mean Scores for Items and Total Test

Item	First Year	Second Year	Third Year	Fourth Year	Total Group
1	6.9	5.7	6.3	5.5	6.1
2	6.6	4.8	5.7	4.8	5.5
3	6.7	5.1	5.7	5.0	5.6
4	4.6	4.1	4.4	3.1	4.1
5	5.8	5.8	5.7	4.9	5.6
6	5.7	5.0	5.3	5.1	5.3
7	5.7	4.6	5.2	5.1	5.2
8	5.7	5.0	5.5	5.3	5.4
9	6.8	5.0	6.1	5.7	5.9
10	6.2	6.3	6.1	5.9	6.1
Total test	6.1	5.1	5.6	5.0	5.5
N	27	15	21	15	78

years of experience and so on for the third- or fourth-year students. The possibility that these students had had previous experience with syllabi of the type and scope as was prepared by the curriculum studied here is highly unlikely.

The data in Table I show that three important aspects of syllabi are rated particularly highly. First, syllabi provide useful study guides for interdisciplinary teaching. Second, syllabi provide more up-to-date information than do texts. Third, syllabi assist students in recognizing important concepts. Thus, one might say that students feel that syllabi provide useful study guides through the complexity of important concepts in medical science as they are developed beyond the content of current texts.

Quite expectedly, the data show that syllabi tend to foster a dependency upon the instructor to organize the material into a logical study pattern. Two-thirds of the curriculum involved was integrated; that is, the teaching was planned and executed by teams of instructors drawn from a spectrum of medical disciplines. For example, in Neuroscience the students receive instruction from neuroanatomists, neurohistologists, neurobiologists, neurochemists, neurophysiologists, neuropharmacologists, and neurologists. The need for a guide through and among these disciplines could not be regarded as unusual.

The results of this study have supported the hypothesis that students do react favorably to the use of syllabi. A logical follow-up of this study would be to determine whether student evaluation of the syllabus for a particular course is related to their achievement. This type of study might also profitably include faculty evaluation of the syllabus.

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Wernicke's Encephalopathy with Intravenous Hyperalimentation: Remarks on Similarities between Wernicke's Encephalopathy and the Phosphate Depletion Syndrome*

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Wernicke's encephalopathy (WE) is characterized by sudden onset of peripheral neuropathy, ataxia, oculomotor abnormalities, amnestic psychosis, and by dramatic improvement with thiamine treatment in some cases and death with hypothalamic, brain stem, and cerebellar lesions in others (1). It is due to thiamine deficiency and, although usually associated with chronic alcoholism, it has also been reported with pyloric stenosis (2), stomach cancer (3), malnutrition (4), starvation diet (5), uremia (6), chronic hemodialysis (7), pregnancy (8), thyrotoxicosis (9), breast feeding (10), soybean diet in infancy (11), and refeeding of prisoners of war (12). Herein we describe an apparent case of WE associated with intravenous hyperalimentation (IVH).

Case Report

HV (BMH #196890), a 61-year-old white woman, was admitted to Blodgett Memorial Hospital on November 20, 1972 for evaluation of persistent vomiting with a 50-pound weight loss in 8 months. Emesis of food particles mixed with bile would occur 5 to 20 minutes post cibum. She had a history of "stomach trouble" since childhood. Past surgery included a cervical interbody fusion, cholecystectomy, and hemorrhoidectomy. She had occasional chest pain considered to be angina. On occasion she used acetaminophen, propoxyphene and nitroglycerin. There was no history of use of alcohol and she had taken no vitamin preparations in the previous year.

She was 5 feet, 2½ inches tall; weighed 107 pounds. The blood pressure was 146/80, pulse 70 and regular, respirations 16 per minute and temperature 37°C. She was thin and her skin hung in loose folds. The PMI was in the 5th intercostal space at the midclavicular line. There were active bowel sounds. A cholecystectomy scar was noted. She was alert and fully oriented. The visual acuity, visual fields, fundi and pupils were normal. Hearing was intact. Gait, power, coordination, and sensation were normal. The deep tendon reflexes were 2 plus and symmetric in the upper extremities, 1 plus and equal at the knees and the ankle jerks were unobtainable. There were no pathologic reflexes.

The CBC, serum proteins, Ca, Pi, cholesterol, uric acid, creatinine, total bilirubin, glucose, alkaline phosphatase, CPK, LDH, SGOT, Na K, Cl and Co_2 were all normal. There was one plus albuminuria. The CSF pressure, WBC, protein and glucose were normal. An EEG and skull and chest x-rays were normal. Both endoscopy and biopsy revealed severe superficial gastritis which was considered psychophysiologic.

She continued to vomit and lose weight and, therefore, on November 29, IVH with Aminosol 5%

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* Abbreviations Used: WE: Wernicke's encephalopathy; PDS: Phosphate depletion syndrome; Pi: Inorganic phosphate; IVH: Intravenous hyperalimentation; ATP: Adenosine triphosphate; 2-3 DPG: 2-3 diphosphoglycerate.

(protein (modified fibrin) hydrolysate, injection, low sodium, USP, Abbott Laboratories) was begun. 40 mEq/L KCl, 40 mEq/L NaCl and 50 mg of 50% glucose were added to the infusion solution daily and it was run at 2400 to 3600 cc per 24 hours. Starting December 4, a multivitamin preparation delivering 10 mg of thiamine per day was added to the IVH solution. By December 5, she had gained 10 pounds. On December 7, with IVH still in progress, multiple small feedings were initiated. On December 8, she complained of numbness of the mouth, hands and feet and IVH was discontinued. By December 9 she was "numb all over," dysarthric and ataxic. The Na, K, Mg, Cl and CO_2 were all normal. The pH was 7.51, pCO_2 37 mm Hg, and pO_2 74 mm Hg.

On December 10, there was scanning speech, intention tremor, inability to sit or stand, and titubation of the head and trunk with attempts to sit. The mental status and cranial nerve functions, including eye movements and pupillary reactions, were normal. Face sensation was not detectably impaired and there were no pathologic reflexes.

Intramuscular thiamine, 500 mg per day, was begun on December 11. By December 12, speech was definitely improved and she was, once again, able to sit unaided. By December 15, the numbness was abating, speech had further improved and the finger-to-nose test was more normally performed. By December 17, she was again able to walk alone. By December 20, her neurologic status was normal, with the exception of still absent deep tendon reflexes and she was discharged. By February 8, 1973, the deep tendon reflexes could once again be elicited. The Na, K, Cl and CO_2 were determined on an every other day basis throughout and were normal. The blood glucose ranged from 120 to 300 mg%. The Pi was normal on admission but was not subsequently determined. Transketolase levels were not obtained. From December 7 through 14, oral and parenteral intake ranged from 350 to 1500 calories per day with an average of 925. From December 15 until discharge on December 20, oral intake was 2000 to 3000 calories per day.

Comment

The case presently reported was adjudged by the neurologic consultant to be reminiscent of "alcoholic-nutritional cerebellar degeneration," a partial form of WE (1). The sudden onset, the symmetric peripheral neuropathy and cerebellar syndrome, the presence of a plausible mechanism, i.e., caloric intake seemingly in excess of available thiamine and finally, an apparent therapeutic response to thiamine, all seem confirmatory. The case of Drenick et al (5) precipitated by renewed caloric intake following a period on a starvation diet is similar.

There are remarkable similarities between reported cases of PDS (Table IV, 13–17) and WE. Silvis and Paragas (17) comment on the similarity of the fatal course of their patient in case #1 to deaths upon refeeding seen in prisoners released after World War II. Such prisoner-of-war deaths were considered to be due to relative thiamine deficiency (12). In the case of Boelens and Brown (13) the "ballismic" movements of the arms and poor sitting balance may represent cerebellar dysfunction, as in the case presently reported. In the PDS, the frequent absence of a therapeutic response to correction of hypophosphatemia (14, 15), followed by recovery upon discontinuation of IVH and resumption of oral feeding, suggests a causal dietary deficiency. However, some patients (13, 15) recover promptly upon correction of their hypophosphatemia. The role of hypophosphatemia per se in the PDS remains to be delineated. Candidates for IVH are often vitamin depleted. Taking into account the thiamine dependence of carbohydrate metabolism, it is understandable how the caloric increment of hyperalimentation might produce relative thiamine deficiency, i.e., a subnormal thiamine/calorie ratio. Moreover, small doses of thiamine, such as the 10 mg per day given our patient, may be less than adequate prophylaxis during IVH. Relative thiamine deficiency was the likely cause in the case here reported.

Thiamine is phosphorylated in all nucleated cells and has a wide range of activity in carbohydrate metabolism. Thiamine deficiency is the cause of WE. Some product, possibly pyruvate, accumulates, producing abnormal effects. Thiamine requirements, which must be computed relative to caloric intake, are .35 to .50 mg/1000 calories (18–20).

Pi, like thiamine, is essential to carbohydrate metabolism. Travis et al (16) concluded that adequate serum concentrations of inorganic phosphate are necessary to the maintenance of intracellular glucose metabolism: In three symptomatic patients the reduction of red cell 2-3 DPG, but not of ATP, was proportional to the left shift of the oxygen-hemoglobin dissociation curve, i.e., to the increase in red cell affinity for oxygen. Two of the three patients had diffuse EEG slowing. Tissue anoxia is postulated as the cause of the PDS.

Others (21) implicate reduced tissue concentrations of ATP. The pathophysiology of the fall of the serum Pi concentrations in the PDS (15, 16) is poorly understood. Renal losses are minimal. The fact that the fall is precipitous and coincides with the start of hyperalimentation, suggests a massive phosphate shift from the extracellular to the intracellular space. There is also a fall in serum Pi with the administration of insulin and with epinephrine as well. Both increase carbohydrate metabolism and attendant phosphorylation. An increase in phosphorylation would be expected both in starved, vitamin-sufficient patients during hyperalimentation and in carbohydrate-sufficient, thiamine-deficient patients during thiamine treatment. There appear to be no published reports specifying inorganic phosphate concentrations during the treatment of thiamine deficiency syndromes.

Hypophosphatemia in chronic alcoholism was the subject of a recent report (22). Three of twelve patients exhibited obtundation, confusion and seizures; however, red cell metabolism was the subject of interest and little was said of the basis of the neurologic symptoms. While the effects of hypophosphatemia on red cell metabolism have been extensively investigated, the consequences in other organs remain largely unknown.

In both the PDS and WE there may be critical disruption of intracellular glucose metabolism productive of similar or identical neurologic syndromes. Alternatively, cases of PDS bearing close similarity to WE may be due to thiamine deficiency; that is, they may, in fact, be Wernicke's encephalopathy.

Summary

Wernicke's encephalopathy (WE) has been reported upon renewed caloric intake following starvation. Similarly, deaths were encountered upon the refeeding of prisoners at the close of World War II. Herein we report the development of WE during intravenous hyperalimentation (IVH).

A 61-year-old woman with persistent vomiting and weight loss was begun on

IVH. On the 10th day she complained of numbness of the hands, feet and mouth and IVH was discontinued. Subsequently she became "numb all over," dysarthric and ataxic and by the 12th day had a severe cerebellar syndrome with head and trunk titubation and inability to sit or stand. Thiamine, 500 mg per day IM was begun on the 13th day. Rapid and complete recovery ensued and she was discharged on the 22nd day.

The caloric increment of IVH with diminution of the thiamine/calorie ratio appears to have produced WE. Similarities between the PDS and WE are noted. There may be a basic disturbance of intracellular glucose metabolism in both. Alternatively, cases of PDS bearing close similarity to WE may be due to thiamine deficiency; that is, they may, in fact, be WE.

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Time Interval to Invasive Mammary Carcinoma in Patients with Atypical Small-Cell Dysplasia and in Situ Carcinoma

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Abstract

Twelve patients who developed invasive carcinomas subsequent to the detection of small-cell mammary dysplasias are presented. No correlation could be demonstrated between the severity of the initially detected lesion and the time to infiltration. The majority of the invasive tumors appeared within the 6- to 15-year period following biopsy. Only 3 cancers developed during the first 5-year period. Prolonged delay may be observed prior to the appearance of an invasive tumor—on one occasion a period of 18 years elapsed. These considerations may influence therapy in the case of an aged patient, or in patients who are suffering from other serious illnesses in addition to their breast lesion.

Invasive carcinoma of the breast supervenes in a small number of patients who have been found to harbor dysplastic lesions of the mammary tubules. This report will analyze the time intervals involved between the initial detection of precancerous changes and the ultimate appearance of an invasive tumor.

Material: Breast biopsies from some 4000 patients, operated on over a 29-year period (1946–1974) have been reviewed, and all patients in whom dysplastic lesions were detected have been placed on a program of annual follow-up. The group is restricted to patients in whom no more than local excision was performed at the initial procedure. Follow-up is maintained, wherever possible, for a period of 20 years subsequent to biopsy. Follow-up is discontinued when the 20-year period has been attained.

Pathology: The pathologic features of small-cell dysplasia and in situ carcinoma have been previously detailed (1, 2) and will only be briefly summarized here. The lesions comprise a morphologically homogeneous group in which a continuous gradation may be traced from an early basal proliferation (Fig. 1), through progressive accumulation of small cells within the acini or ducts (Fig. 2), to the ultimate florid lesion (Fig. 3) in which masses of malignant cells produce gross distension of the mammary channels. The process has been subdivided into 3 morphologically separable stages: grade I (dysplasia), grade II (indeterminate), and grade III (frank in situ carcinoma). The patients have been grouped accordingly. It should be noted that these lesions are preinvasive. Any evidence of infiltration at the time of initial biopsy would exclude the patient from this series. It should, furthermore, be emphasized that the follow-up group includes only patients with small-cell proliferations of solid type. Evaluation of biologic behavior in a group demands morphologic uniformity, and for this reason large-cell lesions, papillary, and cribriform proliferations have been excluded.

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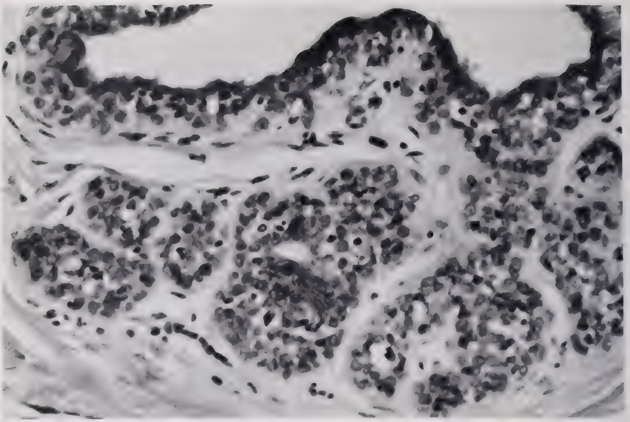

FIG. 1. Group I small-cell dysplasia. Proliferation of small cells produces basally located sheets (H&E x 200).

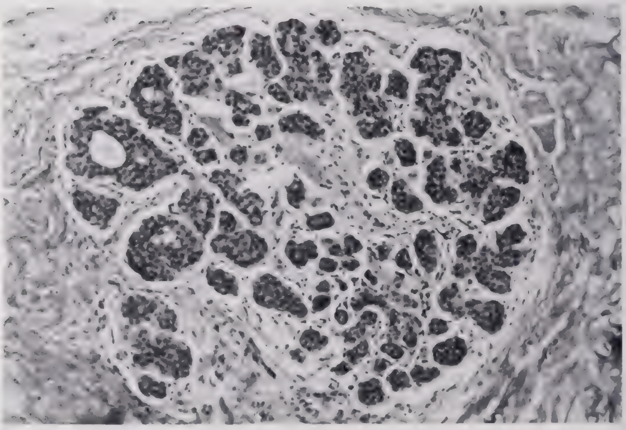

FIG. 2. Group II small-cell dysplasia. Acini are crowded by masses of small cells (H&E x 80).

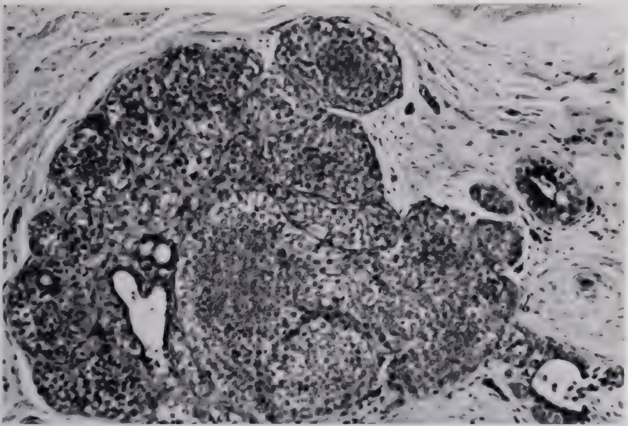

FIG. 3. Group III small-cell dysplasia (florid in situ carcinoma). Acini are distended and globular. Proliferating small cells form solid, homogeneous sheets (H&E x 80).

TABLE I

Interval between Positive Biopsy and the Development of Invasive Carcinoma. Year in Which Invasive Cancer Was Removed.

Year	1	2	3	7	8	11	13	18.
No. of Cases.	1	1	1	3	1	2	2	1.

TABLE II

Interval between Positive Biopsy and the Development of Invasive Carcinoma. Analysis According to 5-Year Periods.

Years	0–5	6–10	11–15	16–20.
No. of Cases.	3 (25%)	4 (33%)	4 (33%)	1 (8%).

TABLE III

Interval between Positive Biopsy and the Development of Invasive Carcinoma. Cases Grouped According to the Severity of the Dysplasia in the Original Biopsy.

Year	Group I No. cases	Group II No. cases	Group III No. cases
1	1		
2		1	
3	1		
7	1	1	1
8	1		
11	1		1
13			2
18	1		

Follow-Up Data: To date, 12 patients have developed invasive carcinomas in the breast harboring the dysplastic lesion.* Time intervals to appearance of the invasive cancer are presented in Tables I and II. These times range from less than 1 year to 18 years. However, the majority of the infiltrating tumors developed within the 6- to 15-year period following detection of the initial lesion at biopsy. Only 1 invasive tumor appeared during the first year subsequent to biopsy, and only 3 during the first 5-year period thereafter.

Table III presents an analysis of the patient group according to severity of the initial dysplastic change. It might have been anticipated that infiltration would supervene more rapidly in patients harboring more advanced lesions at initial biopsy. This has, however, not been the case, and no correlation can be demonstrated between the severity of the initial lesion and the time interval to infiltration.

Comment

The time interval to development of an invasive tumor in patients with small-cell dysplasia or in situ carcinoma is unpredictable. It has not been possible to correlate this interval with the severity of the initial lesion as observed on biopsy. The majority of invasive tumors develop after a five-year period has elapsed, and, in some instances, prolonged latency may be encountered. Thus, one patient manifested an infiltrating tumor 18 years subsequent to detection of the initial lesion. We have, furthermore, observed another patient in whom a Grade I lesion was detected at the first excision. Biopsy after a period of 16 years revealed, again, a dysplastic lesion that exhibited only slight progression from the original degree of change.

It is felt that these considerations may influence therapy in patients who are aged, or who are suffering, concurrently, from other serious illnesses in addition to their breast lesion.

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* Subsequent to the preparation of the manuscript 2 further invasive carcinomas have been recorded—one 5 years after initial biopsy of a Grade III lesion, and one 13 years after biopsy of a Grade I lesion.

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The Role of Psychological Factors in Reversible Drug-Related Dystonic Reactions*

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Abstract

The predisposition to develop reversible drug-related dystonia and the role of anxiety in precipitating dystonic crises is reviewed. Five cases are presented in which the dystonic reaction develops in relation to emotional stress. Hysteric and paranoid traits may contribute a propensity to the development of the syndrome.

The reversible dystonia induced by psychotropic drugs consists of tonic contractions of the voluntary muscles of the eyes, face, neck, trunk, or extremities. Dystonia is one of several extrapyramidal syndromes associated with the psychotropic medications and is distinguished by reversibility and occurrence early in the course of treatment (1). In a study of a large hospital population during prolonged treatment with phenothiazines 38.9% of patients showed extrapyramidal syndromes including 2.3% of the total population with dystonia, while 61% remained free of extrapyramidal disorders (2). The factors responsible for the susceptibility to the development of reversible dystonia require clarification.

The occurrence of extrapyramidal disorders appears to be determined partly by the dosage and pharmacologic properties of psychotropic medication. A number of studies have shown the propensity of piperazine phenothiazine and butyrophenone drugs to induce extrapyramidal disorders (2, 3). An idiosyncratic neurologic sensitivity has been suggested to account for the susceptibility of individuals who develop extrapyramidal symptoms with low does of medication (2, 3). A metabolic factor, hypocalcemia, related to hypoparathyroidism has been observed to predispose individuals receiving psychotropic drugs to develop dystonia (4). Furthermore, the hypothesis was presented that genetic factors may be involved in sensitivity based on a higher incidence of Parkinson's diseases in relatives of afflicted patients (5).

Another determinant of susceptibility to dystonia may be the role of personality and psychological factors. Although early reports of phenothiazine treatment suggested that psychological processes may induce dystonia and account for reversibility of symptoms, little exploration of this aspect is present in the psychiatric literature (6). The influence of emotional stress and personality traits in the precipitation of dystonic reactions is illustrated in the following examples. The patients were treated in the Psychiatry Department

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* This paper is a prizewinner in the 1975 competition for resident research of the New York City District Branch of the American Psychiatric Association.

of the Mount Sinai Hospital during a ten-month period and were studied individually by the author, who was associated in their treatment.

Case Reports

Case No. 1. A 24-year-old woman was hospitalized with symptoms of depersonalization and ideas of being controlled by other individuals. She had a dramatic, at times agitated manner, and spoke of her family with idealized affection. Her emotional concerns focused upon a younger brother toward whom the patient felt a protective responsibility.

On the third day of treatment with haloperidol 2 mg three times daily she spoke during an interview of feeling responsible for her sibling but went on to describe incidents in which he provoked her to anger. When encouraged to continue she complained of tightening in her throat and appeared anxious. Within minutes the spasm had progressed to trismus, preventing further speech. It was suggested that the patient relax and avoid thoughts of her brother, an effort which brought about a return of speech and a reduction in anxiety. The symptoms reappeared when thoughts and feelings of resentment recurred. This example demonstrates the appearance of dystonia during a state of anxiety related to the expression of resentment associated with conflict about the idealized view the patient had regarding her brother.

Case No. 2. A 52-year-old housewife and former professional dancer was hospitalized with ideas of being spied upon. She was youngest of 8 children and characterized herself as immature. Her illness developed after her husband was diagnosed as having cancer. The patient had suffered a prior psychotic illness when her mother died of a malignancy. In the hospital she received haloperidol 2 mg three times daily for four days. When interviewed by her doctor she remarked that the room was bugged and she seemed quite anxious. She was encouraged to speak about her husband's illness but was reluctant. Within several minutes her jaw became stiffened and she was unable to speak. The patient's fear regarding her husband's condition was extreme and her identifications with him became more evident with the development of somatic hysterical symptoms and a preoccupation with symptoms of malignancy.

Case No. 3. A 13-year-old girl was seen following the death of a school friend. The patient developed hallucinations and expressed the wish to join her schoolmate in heaven. She was childish and her illness showed aspects of a hysterical psychosis. On the eve of the friend's funeral, while receiving thioridazine 25 mg three times daily, she developed torticollis. She expressed panic regarding the funeral and fear of the dead girl's spirit appearing during the ceremony. The patient's symptoms were relieved with suggestion and advice to avoid the ceremony in the morning. The dystonia occurred in a state of emotional conflict and anxiety associated with the funeral.

Case No. 4. A 37-year-old married woman was hospitalized with the belief that her thoughts could be read. She had a seductive manner and appeared considerably younger than her age. Following previous illnesses, her household responsibility was decreased, allowing the patient to maintain a fragile adjustment. This woman's mother-in-law became ill and requested nursing care from the patient. This request resulted in the patient's decompensation and hospitalization.

On the eighth hospital day while being treated with fluphenazine 2 mg four times daily, she received a call from her mother-in-law. The patient became distressed when her mother-in-law asked for assistance again over the telephone, disregarding the patient's own condition. Within minutes the patient's gaze was drawn upwards and her eye muscles were in spasm. This woman expressed much resentment toward her mother-in-law.

Case No. 5. A 20-year-old student was admitted to the hospital with the belief that people were following and conspiring against him. He had delusions of possessing espionage materials. His illness developed in a foreign country under circumstances of romantic disappointment. He was treated with chlorpromazine 100 mg four times daily. The patient was asked about his romantic affair and became evasive. It was apparent that the question caused anxiety and within moments his speech slurred. This reaction progressed until his facial muscles were distorted in dystonia. The reaction ceased with the termination of the interview.

Discussion

The clinical cases presented demonstrate the development of dystonia in relation to emotional stress. In each case the patient was treated with moderate doses of psychotropic medication and a reversible dystonia appeared following an emotion-laden situation in an interview or life event. The emotional stresses included illness, the expression of resentment or loss, or the frustration of needs. The stress for each patient seemed specific and related to the psychological vulnerability of the subject.

The patients in this report showed trends in their personality which consisted of emotional immaturity, dramatization, and dependency similar to traits described in hysteric character disorders. The occurrence of dystonia in hysteric character type suggests that it is a related phenomenon to somatic conversion.

The observations of the association of emotional stress and personality with reversible dystonia are in accord with those of Delay and his coworkers (6). He described the emotional state seen in individuals with dystonic symptoms induced by chlorpromazine to be one of exaggerated emotional lability. Delay suggested a resemblance to hysteric phenomena by describing the drug-related dystonia as *crises hysteriformes*. This syndrome was believed to be similar to the neuromuscular spasms in postencephalitic conditions which are precipitated by emotions.

More recent work has questioned the relation of reversible dystonia to hysteria. In examining this matter Simpson and Angus note the difficulty inherent in demonstrating the action of psychological factors (9). They present a theory, however, which views drug-related dystonia in a psychosomatic context. Other recent articles emphasize a concern for diagnosis and therapeutic intervention, and distinguish reversible dystonia from hysterical conversion symptoms (10, 11).

The psychopathology of the patients studied in this report showed a predominance of paranoid symptoms such as delusional ideas, ideas of control, or suspiciousness. Further paranoid attitudes were observed in a reluctance to accept medication or to establish a working relationship with the physician. The propensity of paranoid patients to develop akathisia when treated with psychotropic medication has been viewed as being responsible for their reluctance to accept treatment with drugs (12). Paranoid ideation may influence an individual's susceptibility to develop extrapyramidal reactions.

The relation of psychological phenomena to neural activity may be considered in terms of similarities in emotional excitation and brain excitation (13). Thus, emotional states characterized by increased reactivity such as anxiety are associated at the physiologic level either with increased excitation or disinhibition in inhibitory pathways. The extrapyramidal system is the pathway for the motor discharge of such excitation or disinhibition. It is connected by neural circuits both to higher cortical and lower motor centers. Anxiety states, even in the absence of pharmacologic substances, are associated with increased motor activity, presumably on the basis of cortical inputs to the extrapyramidal system (14). The presence of psychotropic agents induces a greater instability in the extrapyramidal system, as do a variety of drugs and physical injuries. This instability may result in a tendency to hyperkinesis or parkinsonism, depending upon the balance of specific factors. These case reports suggest that the drug-related tendency to hyperkinesis manifest in dystonic symptoms is accentuated in patients with specific emotional stress and personality type.

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Signet Ring Verruca

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Warts probably are one of the most common afflictions of man. The various histologic variants that are recognized (verruca vulgaris, verruca plana, and condyloma acuminatum) have well-defined, distinctive microscopic patterns which need not be detailed here. The verruca plantaris, in my opinion, should not be considered a histologic variant and basically represents the common wart located on the sole. During the past year, I have observed what I believe to represent the emergence of a new histologic variant which is the subject of this report.

Materials and Methods

Fourteen lesions were biopsied from ten patients (five from case 10) and were fixed in Bouin's solution, routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, periodic acid-Schiff reaction (PAS) with and without diastase digestion, alcian blue (pH 2.5) and the Feulgen reaction for deoxyribonucleic acid (DNA).

The clinical data are summarized in Table I. All lesions were verrucous, skin-colored papillomas which ranged in size from 0.4 to 1.5 cm in maximum dimension. Multiple lesions were present in two cases (4 and 10), two others located on the same index finger in case 4 not being biopsied. There was a recurrence of one lesion following previous removal by cauterization (9). Although the number of cases is too small for the determination of sex preponderance, there seems to be no significant predilection. All lesions except one involved the hand (93%), 57% of them occurring on the index finger. In all instances, removal was by surgical excision with cauterization of the base and there have been no recurrences thus far.

Examination of each case by light microscopy disclosed remarkably similar histologic features in all. These were characterized by a considerably papillomatous epidermis showing variable degrees of hyperkeratosis, parakeratosis, hypergranulosis and acanthosis. The rete ridges at the periphery were bent inward and the subepidermal zone showed prominent vascular ectasia (Figs. 1 and 2). Many of the keratinocytes within the upper malpighian and granular layers were enlarged, with most of the nuclear and cytoplasmic material appearing compressed, crescent-shaped and eccentric, with the remainder of these cells being composed mainly of a single large either clear or faintly eosinophilic vacuole, resembling signet rings (Fig. 1 through 4). Although in some of these keratinocytes, the vacuoles appeared to be

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TABLE I

Signet Ring Verruca

Case	Age	Sex	Location	Size*	Duration	Clinical Impression
1	60	M	Index Finger	1.5 cm	24 mo	Verruca Vulgaris or Fibroma
2	41	F	Index Finger	0.8 cm	6 mo	Verruca Vulgaris
3	68	M	Index Finger	0.4 cm	4 mo	Verruca Vulgaris
4	16	M	Index Finger	0.5 cm	2 mo	Verruca Vulgaris
5	28	F	Dorsum of Hand	1.0 cm	18 mo	Verruca Vulgaris or Seborrheic keratosis
6	50	M	Axilla	1.0 cm	12 mo	Skin tag
7	34	M	Index Finger	0.7 cm	9 mo	Verruca Vulgaris
8	31	M	Index Finger	1.2 cm	12 mo	Verruca Vulgaris
9	21	F	Thumb	1.0 cm	9 mo	Verruca Vulgaris
10	48	F	Index Finger (2) Middle Finger (2) Thumb	0.4 to 0.8 cm	8 mo	Verruca Vulgaris (5)

* Size represents maximum dimension.

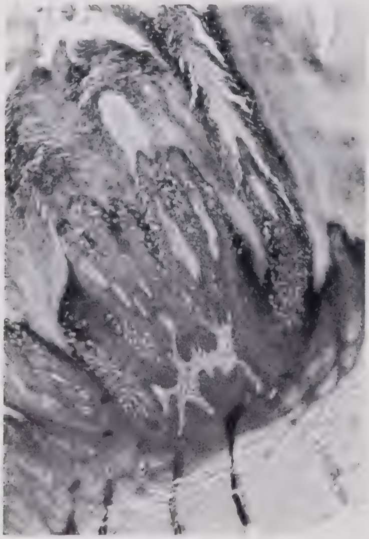

FIG. 1. Scanning view of case 2 showing verrucous nature of lesion as well as hyperchromatic nuclei within thickened horny layer, conspicuous signet ring cells within granular and malpighian layers, and vascular ectasia of papillary cutis (H & E x 25).

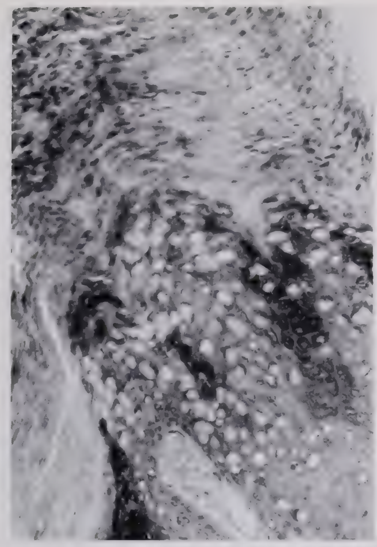

FIG. 2. Higher magnification of Figure 1, detailing features described (H & E x 100).

surrounded by a rim of compressed nuclear chromatin (Fig. 3), suggesting that these vacuoles might have an intranuclear origin with eventual rupture into the cytoplasm, it seems more likely that these represent portions of intracytoplasmic vacuoles surrounded in part by compressed rims of nuclear chromatin. Enlarged, coarse keratohyaline granules were often observed within these vacuolated cells. Large, hyperchromatic, generally ovoid or round feulgen-positive nuclei were prominent within the thickened horny layer overlying these vacuolated cells (Figs. 1 and 2). Weakly feulgen-positive nuclei could also be demonstrated within the nuclei of some of these vacuolated cells. A number of the keratinocytes within the lower malpighian layers had enlarged hyperchromatic nuclei with and without vacuole formation, some of these cells appearing binucleated or multinucleated (Fig. 4). No mucopolysaccharides (acid or neutral) could be demonstrated within these vacuolated cells.

Comment

The presence of cells resembling signet rings (thin-banded rings with a seal at the margin) within the epidermis has been previously reported (1). The absence of demonstrable mucin within these cells would exclude a muciparous tumor. Paranuclear vacuolization in keratinocytes has been reported following suction (2) and other forms of epidermal injury with the production of crescent-shaped nuclei and compressed cytoplasms simulating signet rings.

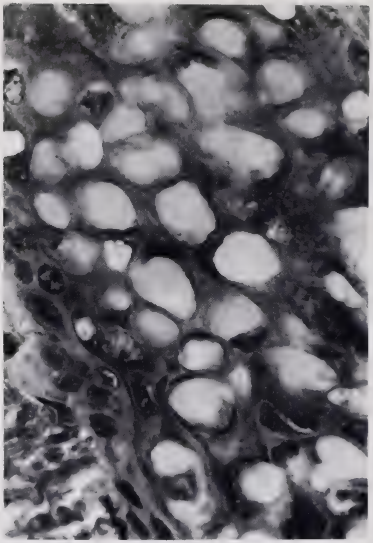

FIG. 3. High magnification of case 2 detailing vacuoles, some of them seeming to be surrounded by a rim of compressed nuclear chromatin (H & E x 400).

The clinical and microscopic features of the cases under discussion would exclude such causes. Although molluscum contagiosum shows prominent cytoplasmic viral inclusions imparting a signet ring appearance to the infected cells, the clinical picture, histologic configuration, and microscopic features of the signet ring cells would negate this diagnosis. The combined clinical and histologic features of the present cases are strongly indicative of a verrucous papilloma of viral origin. The noticeable difference between this lesion and other verrucae is the transformation of epidermal keratinocytes into conspicuous signet ring cells.

What is the nature of the vacuolar material? It does not appear to be mucin or virus DNA. Edema and/or a viral degeneration product, perhaps lipid-containing, stemming from an intranuclear virus seem to represent the most likely possibilities. Since I have observed all of these cases only during the past year (representing nine percent of all verrucae observed that year), it is suggested that, in addition to the other three well-recognized histologic variants, this represents the emergence of a fourth histologic variant, probably caused by the same virus. It appears to have a strong predilection for the hand, most notably the index finger. I was not able to perform electron microscopic studies, which would probably establish the cytoplasmic origin of these vacuoles leading to signet ring formation. I hope to report such studies at a later date.

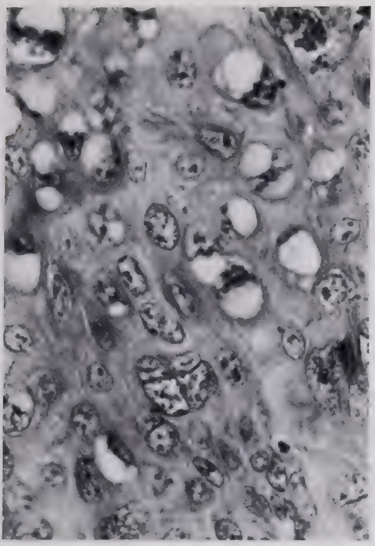

FIG. 4. High magnification of case 2 showing binucleated and multinucleated cells as well as vacuole formation within lower malpighian layers (H & E x 400).

Summary

Fourteen verrucae containing conspicuous signet ring cells have been observed during the past year. They showed a striking predilection for the hand, most notably the index finger. The vacuoles within these signet ring cells are probably entirely intracytoplasmic and are most likely composed of edema and/or a viral degeneration product, perhaps lipid-containing. It is suggested that this lesion represents the emergence of a fourth histologic variant of wart, probably caused by the same virus responsible for other verrucae.

Acknowledgments

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Raynaud's Syndrome following Self-Administered Intraarterial Barbiturate

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Numerous complications and consequences of accidental intraarterial injection of barbiturates have been described. Originally these were mostly related to the field of anesthesiology (1, 2). In recent years the increasing number of drug addicts have provided experience in this field, ranging from transient symptoms and circulatory changes to severe ischemia and loss of fingertips and forearm (3–7). The appearance of localized Raynaud's phenomenon following intraarterial barbiturate injection has so far not been described.

Case Report

A 20-year-old man was referred for consultation with a diagnosis of Raynaud's syndrome of his right hand. He gave a history of a severe crush injury to his right hand seven months previously, resulting in immediate severe pain, swelling and bluish discoloration. When these symptoms and physical changes subsided several weeks later, he began to notice coldness, numbness and pallor or cyanosis of the thumb and index finger on exposure to cold. In very cold weather these changes extended to all fingers of that hand. The right and left hand had previously been completely normal, and there was no personal or family history of Raynaud's disease. Physical examination at that time revealed a normal brachial, and doubtful radial and ulnar pulses. Digital artery pulsations were not palpable on either hand. The affected thumb and index fingers were minimally cyanosed, and showed very slight tissue atrophy. Immersion of the hand in cold water reproduced discoloration and numbness of the thumb and index.

Routine investigations were performed to exclude cervical rib, collagen disease, vasculitis or increased blood viscosity—all with completely normal results. In order to elucidate the somewhat puzzling clinical picture, a brachial arteriogram was performed (Fig. 1). This demonstrated a rather small radial artery which was occluded at approximately mid-forearm level. The ulnar and interosseous arteries were patent and somewhat hypertrophied. It was only at a subsequent office visit that the patient confessed that his original story was untrue, and that on the day of the "accident" he had actually "shot a barbiturate"—he had apparently dissolved an unidentified barbiturate capsule in a spoon, drawn the fluid into a syringe and injected himself in the forearm with the solution. He thought he had noted an unusually powerful return of blood into the syringe, and he felt extremely severe pain in the arm and hand immediately upon completing the injection.

Comment

This patient developed distal radial artery thrombosis following intraarterial barbiturate injection. Presumably sufficient collateral circulation developed to allow recovery of the ischemic areas, but not sufficient to prevent the appearance of a localized Raynaud's phenomenon under the stress of low temperature. Cervical sympathectomy was recommended but the patient refused surgery.

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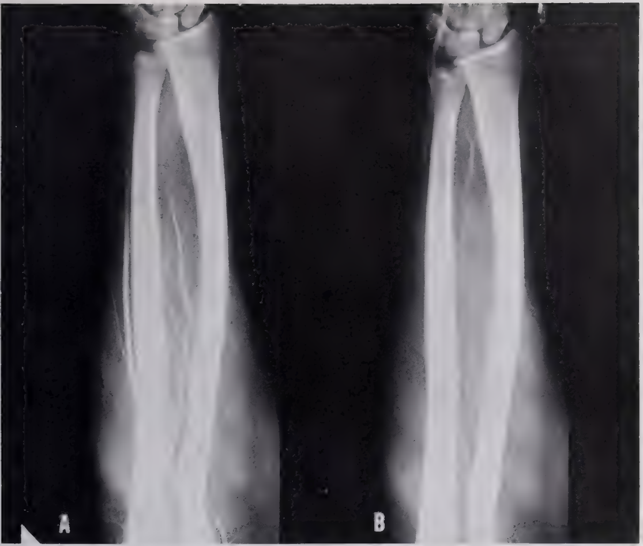

FIG. 1. Brachial arteriogram showed slight hypertrophy of ulnar and interosseous arteries. The radial artery is occluded just above the midpoint of the radius. (a) early (b) late

Summary

A patient is reported who developed localized Raynaud's phenomenon of the thumb and index finger some months after self-injection of a barbiturate. No record of this complication has been found in the literature.

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Cystic Diseases of the Liver

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Cystic diseases of the liver are uncommon and are the primary cause of symptoms only on rare occasions. This paper presents two cases of polycystic disease of the liver and reviews the classification of cystic diseases of the liver.

Case Reports

Case 1 (#077218): The patient, a 65-year-old woman, was admitted to The Mount Sinai Hospital with a week's history of dull right upper quadrant pain.

The patient was being treated for hyperthyroidism. Six weeks prior to admission she was noted to have a smooth nontender right upper quadrant mass (presumed to be liver) two fingerbreadths below the right costal margin. In the weeks prior to admission the mass increased in size to five fingerbreadths and began to cause a continuous dull pain. The patient was otherwise asymptomatic.

On admission to the hospital the patient's temperature was 98.6°F, blood pressure was 120/70, pulse 100, respirations 16/minute.

On examination the patient had a diffusely enlarged thyroid. Her abdomen was soft. A firm, slightly tender, smooth and somewhat mobile mass occupied the right upper quadrant extending to five fingerbreadths below the costal margin.

Her hemoglobin was 11.8 gm %, white blood count 5,200, bilirubin 1.0, alkaline phosphatase 320, blood urea nitrogen 10, and urinalysis normal.

Flat plate of the abdomen revealed a soft tissue mass in the right upper quadrant. Oral cholecystogram, intravenous cholangiogram, and intravenous pyelogram were all normal. Upper gastrointestinal series and barium enema revealed an extrinsic mass displacing the duodenum medially (Fig. 1) and the hepatic flexure and transverse colon inferiorly (Fig. 2). Celiac angiography demonstrated a space-occupying lesion in the right lobe of the liver with no tumor blush.

Prior to laparotomy the patient's hyperthyroidism was treated with Tapazole and radioactive iodine.

At laparotomy a polycystic liver was found with evenly distributed cysts measuring about 1.0 cm each and one large cystic mass (15 x 15 cm) which extended from the inferior aspect of the right lobe of the liver. No cystic disease of the kidneys or pancreas was present.

A partial right hepatectomy was performed excising the large cyst. The patient's postoperative course was uneventful. Histologically the tumor was a bile duct hamartoma. The patient has remained well for 4 years.

Case 2 (#822068): The patient, a 51-year-old man, was admitted to The Mount Sinai Hospital with pyloric obstruction secondary to a chronic duodenal ulcer. A vagotomy and gastroenterostomy was performed. At laparotomy a polycystic liver and polycystic kidneys were noted. Liver biopsy was read as "bile duct cysts with chronic portal inflammation and fibrosis." The patient has remained well for 3 years.

Discussion

Cystic diseases of the liver are uncommon. The following is a compilation of several classifications (1, 2):

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Cystic Disease of the Liver

- I. Congenital cystic disease of the liver
  - A. Solitary cyst of the liver
  - B. Polycystic liver disease
- II. Acquired cystic disease of the liver
  - A. Traumatic liver cyst
  - B. Inflammatory cysts of the liver
    - 1. Retention cyst due to inflammatory obstruction of bile ducts
    - 2. Hydatid cyst of the liver

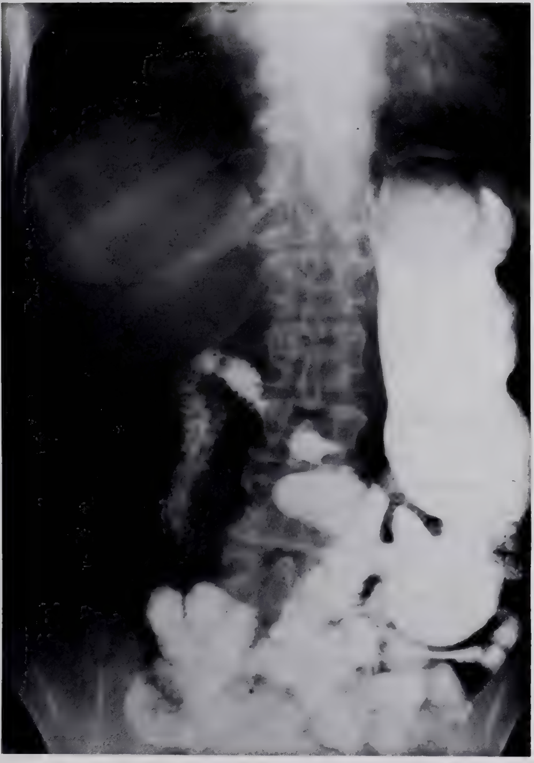

Fig. 1. Upper gastrointestinal series. The duodenum is displaced medially by an extrinsic right upper quadrant mass.

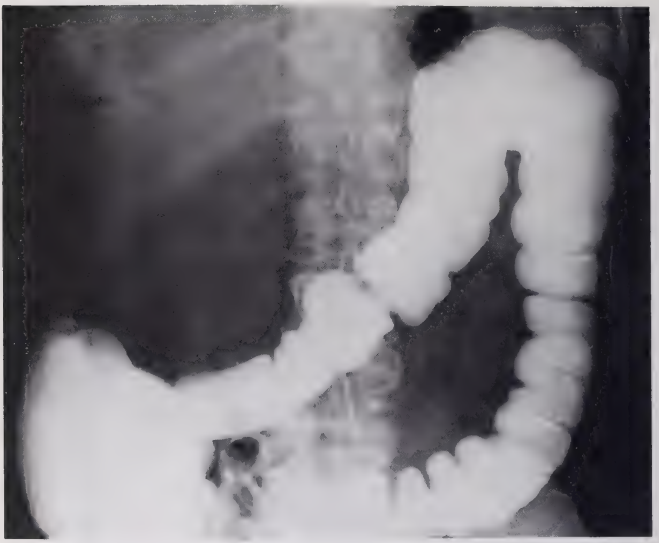

FIG. 2. Barium enema. The hepatic flexure and the transverse colon are displaced inferiorly by an extrinsic right upper quadrant mass.

C. Neoplastic cysts of the liver
  1. Primary tumors
    a. dermoid or teratoma
    b. angioma or hemangioma
    c. bile duct cystademona
    d. hamartoma
      i. mesenchymal hamartoma
      ii. bile duct hamartoma
    e. retention cyst due to neoplastic obstruction of the bile ducts
    f. lymphatic cysts
  2. Secondary tumors
    a. cystic degeneration of a metastatic neoplasm.

The cysts that are found in congenital solitary cystic disease and polycystic disease are essentially the same. Both represent an embryological maldevelopment. An abnormal extension takes place of the normal process of degeneration of small intrahepatic bile ducts. Overproduction of bile ducts in the 10–23 mm embryonic stage apparently occurs. While the rest of the liver is developing normally the excess bile ducts manifest changes characterized by distortion, segmentation, and cystic formation. The first generation of bile ducts showing these changes is ultimately resorbed. In the cystic liver, however, the degeneration is abnormally extended and the cystic formation persists (3).

Similar embryologic maldevelopment can occur in other organs. About half of the patients with polycystic disease of the liver have associated polycystic disease of the kidneys. Conversely about one-third of the patients with polycystic disease of the kidneys have associated polycystic disease of the liver. Polycystic changes in the pancreas, spleen and lungs may also be associated (4, 5).

In addition, a large percentage of patients with polycystic disease of the liver have associated multiple cerebrovascular aneurysms. It has been suggested that polycystic disease of the liver, polycystic disease of the kidney and congenital aneurysms of the basilar artery may be transmitted by a single dominant gene with both variable penetrance and expressivity (6). Polycystic disease both of the liver and kidney may be familial (7, 8).

Polycystic disease of the liver is slightly more frequent than solitary cystic disease of the liver. Of 499 cases of cystic disease of the liver, 219 were solitary cysts, including 20 which were solitary but multiloculated (9).

The solitary cysts are usually small but may reach several centimeters in diameter. On rare occasions the cysts have reached enormous proportions—the largest being greater than 4 gallons, almost 17,000 cc (10).

Females are affected almost four times as frequently as males. The most common age group for discovering the condition is 20-50. Occasionally cases are discovered in childhood or in old age, the oldest being 82 years of age.

Typically the cysts have a cuboidal or columnar epithelial lining and a circumscribed connective tissue wall. Frequently the cysts contain biliary duct lining of obstructed bile ducts. In many instances the cyst lining contains all cell types. If the cyst has an epithelial lining it may be described as a "true" cyst as compared with a "false" cyst lined by fibrous tissue (11).

Solitary cysts in some instances may represent multiple cysts which have coalesced into a unilocular or multilocular cyst (9, 11).

The cysts are usually round or ovoid and are well encapsulated and circumscribed. The liver architecture surrounding the cyst appears normal. However, microscopic examination usually shows evidence of mild cholangitis and leukocytic exudation. The intrahepatic part of the cyst may contain segments of bile ducts and dilated vascular channels. In the larger cysts the fibrous wall is thicker and often shows lymphocytic infiltration (12).

The cystic fluid is either serous, bile-colored, or mildly serosanginous with a specific gravity between 1.010 and 1.022. The cyst fluid contains albumin, mucin, bilirubin, chlorides, glucose, and cellular debris (10).

The right lobe of the liver is affected twice as often as the left lobe both in solitary cystic disease and polycystic disease. The solitary cyst is located most frequently just below Glisson's capsule (10). If the cyst is large and symptomatic it most frequently protrudes, as in our patient, from the inferior aspect of the right lobe. With polycystic disease the cysts are likewise most commonly located just below Glisson's capsule (13, 14).

Most solitary cysts of the liver are asymptomatic. At the Mayo Clinic in 27 years only 82 cases were reported and of these only 15 were symptomatic (15).

Polycystic disease of the liver is noted infrequently during life. Eleven cases in 6141 autopsies were reported (4). The cysts varied from 2 or 3 to multiple in both lobes. They also varied from microscopic to 1000 cc or more. Noncystic liver architecture is normal or shows mild cholangitis and fibrosis (12).

Usually neither solitary cystic disease of the liver nor polycystic disease of the liver causes symptoms. When symptoms are present they are usually right upper quadrant pain or pressure or a right upper quadrant mass. If the cysts are large enough to cause symptoms, four of five are palpable (10). Jaundice is rare (10, 16).

Diagnosis may be difficult. X-rays are nondiagnostic. Liver function tests are usually normal or only mildly elevated. Liver scan and celiac angiography may be normal or may show a space-occupying lesion (17). Needle biopsy is helpful if the cysts are not too large. A portion of the cyst wall may be obtained (18). If the cyst is large, needle biopsy may cause cyst rupture, bleeding and peritonitis and is generally not indicated. Peritoneoscopy, if feasible, and laparotomy are diagnostic.

Treatment is usually conservative and nonsurgical. Emergency surgery is indicated only for cyst rupture, hemorrhage, torsion or strangulation. All of these are rare. Elective excision when necessary is preferred by most authors (20).

Acquired cystic disease of the liver secondary to trauma is rare and almost never is responsible for a primary condition that requires surgery. When present it is usually due to automobile accidents, steering wheel injuries, etc.

Retention cysts of the liver due to inflammatory obstruction of the bile ducts (cholangitis or portal cirrhosis) occurs occasionally, but the cysts are almost always small (11).

Hydatid cysts, on the other hand, are the most common cystic disease of the liver. They are secondary to the lodgement in the liver of the larval form of the dog tapeworm, Taenia echinococcus. The disease is found where men live in close contact with dogs (e.g., German shepherds). It is rare in this country, but common in South America, Greece and many other countries. Only 30 primary cases have been reported in the United States whereas over 6,000 cases have been reported in Greece (21).

Once the larvae spread beyond the intestines the liver is frequently involved. The cyst walls are hyaline laminae lined by germinal cells from which the "daughter" cysts grow. Hooklets and scolices may be identified in the cyst wall or in its contents by microscopic examination. Old cysts with dead parasites contain yellowish-gray putty-like material (21).

The cysts are characterized by large fibrous envelopes (the false cyst) containing a soft lining or membrane (the true cyst). Calcification of the cyst wall frequently occurs and may be identified on x-ray. Surgical treatment when indicated is to aspirate the cyst through a hollow needle and then carefully to open the false cyst and dissect out the collapsed true cyst. The true cyst should be excised intact. The false cyst may or may not be excised. If it is not it should be closed over drains (22).

Teratomas or dermoid tumors of the liver are very rare and are identical with lesions encountered more commonly elsewhere. They are somewhat more common in infancy and childhood. When present at birth they are relatively benign and grow slowly (23).

Angiomas and hemangiomas are the most common liver tumors. They are more common in the liver than in any other internal organ, and may be either single or multiple. Only rarely do they cause symptoms. All are benign. At Memorial Hospital, of 106 patients with hemangiomas of the liver, 84 were found at laparotomy for other reasons. The remaining 22 were giant hemangiomas, of which 18 were symptomatic. Symptoms consisted of right upper quandrant pain or pressure. Liver function tests are usually normal, but there may be mild bilirubin elevation. X-rays are nonspecific, although a rare tumor may have diffuse, minute calcifications (24).

Angiomas or hemangiomas tend to be soft or cystic, red to purplish in color and are lobular or irregular in shape. The cyst contents are blood or bloody fluid. They are not usually confused with solitary cysts or polycystic disease of the liver. They apparently grow for a limited period of time and then frequently regress, obliterating the cavity, leaving the so-called sclerosing hemangioma. When accidentally found during surgery they are frequently biopsied to rule out metastatic liver disease (24).

A rare diffuse condition of angiomatoid changes in the liver parenchyma is called peliosis hepatis (25).

Cavernous hemangiomas are blood-filled cysts which may be associated with angiomas of the skin or gingiva. Most are circumscribed, a few mm to several centimeters in size, red to purple in color and located just below Glisson's capsule. Microscopically they are large blood-filled spaces lined by a single layer of epithelium and separated by connective tissue that often has a myxomatous appearance. Treatment is unnecessary unless rupture occurs (26, 27).

Bile duct cystadenomas are an extremely rare variant of bile duct adenomas. Instead of tumors consisting of bile duct epithelium in multiple acinar structures, the cysts are lined by mucin-secreting columnar epithelium and the connective tissue stroma is quite cellular. They may reach a diameter of several centimeters (28).

Two types of cystic hamartomas are recognized in the liver.

Mesenchymal hamartomas are red-to-purple, cyst-like lesions which most often arise from the lower margin of the right lobe of the liver in the first two years of life. They tend to grow rapidly and accumulate fluid in the cyst-like areas. Microscopically they are composed of relatively acellular collagenous connective tissue, remnants of bile ducts and fluid-filled spaces. They probably represent an embryologic maldevelopment of mesenchymal tissue in the fetus or newborn (23, 29).

Bile duct hamartomas are cyst-like lesions which contain clear to bile-stained fluid and are lined by bile duct epithelium. The capsule is usually fibrous and may contain blood vessel remnants. It is suggested by some authors that solitary cystic disease of the liver and polycystic disease of the liver may represent either single or diffuse bile duct hamartomas. As noted earlier, remnants of all types of bile duct elements are frequently found in these conditions (30).

Rare cases of liver cysts secondary to neoplastic disease are reported. These include bile duct papillomas. In these cases the cyst rather than the tumor is the presenting sign.

Lymphatic cysts are due to obstruction or congenital dilatation of the liver lymphatics and are usually on the surface of the liver (31).

Cysts which result from degeneration and necrosis of metastatic liver neoplasms are actually "pseudocysts." The primary tumor may be either carcinoma or sarcoma (32).

Summary

Two cases of polycystic disease of the liver are presented. One patient has a large symptomatic cyst of the right lobe requiring surgery. The etiology and classifications of cystic disease of the liver is reviewed.

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The Doctor's Image: An Approach to a Study of a Universal Ambivalence*

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Pressures are increasing at many levels and criticism of practically every component of health care delivery has become a matter for discussion and activism. No matter what else is under discussion, the role of the doctor is at the core. For the most part, the doctor's image is a negative one.

If one restricts oneself to the literature, it might seem that such criticism has a great deal of validity and the evaluations seem to relate altogether to the current situations. However, even the most superficial reading of history provides us with a context and matrix which demonstrates that the basic attitudes to the healer in all cultures seem to be similar in principle. This presentation is an attempt to study the doctor's image and the ambivalence attached to it as seen through the historical perspective.

Health and disease have always been of fundamental importance to mankind; and speculations, hypotheses and theories relating to these matters have always been encumbered, in direct proportion to our ignorance, with supernatural connotations. Health as a blessing, and illness as a punishment, are to this day beliefs that are present even in the most advanced of Western civilizations. The interpretation of the phenomena of mental disease has been particularly contaminated by such notions.

Fear of illness and dying erects religious customs around illness, death and the healer, and fosters ambivalent attitudes toward him. These attitudes in part can be explained by psychoanalytic concepts.

The practitioner, by virtue of his responsibility for practical action, assumes a degree of vulnerability. What he gains in gratitude he may lose in reproach for failing to work a miracle. Furthermore, the healer is seen as a magician who can *cause* illness and death as well as *cure*. Thus, the unconscious hostility, aggression and death wishes toward the recently dead, which are manifestations of the ambivalence of the bereaved, are displaced to the healer.

This situation is reinforced by the layman's ignorance of what constitutes a doctor's training and day-to-day activities and by his limited and often distorted view of the physician's role as presented in the media. The inherent ambivalence perhaps is best described by the following paragraph from the introduction to *The Doctors* by Martin L. Gross:

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> It is not the purpose nor is it within the power of this book to destroy the reputation of the American doctor. It is not easy to undertake an analysis of the physician soon after one's own child has received a polio vaccine from the hand of a busy pediatrician, or after visiting a convalescent in the hospital, ruddy-faced and well after a successful operation. No one is immune to the fear of illness or death. Having learned—from day to day, through word and picture—that the doctor of medicine may be the only barrier between self and eternity, we find it painful to try to see the modern doctor as he really is. We have taught ourselves, instead, to view him through a prism of fear or hope.

The author throughout the book seems to always prefer a pejorative term to a relatively neutral one. The titles of the chapters themselves indicate his attitude: "Man of Paradox," "Arrogance," "Availability and Assembly-Line Medicine," "The Fallacies of Doctor Erudition," etc.

Gross cites various polls regarding attitudes toward physicians indicating that whereas many people believe that "the good qualities of doctors seem to outweigh their faults by a rather substantial margin," nevertheless there were many dissenters; some felt that the doctors did not take a personal interest, others that they liked to dispense medicines even when they were not needed. Gross concludes that sick people like the doctor least, that the aged respect the doctor less and the young and healthy "may still naively view the physician as a 'miracle worker.'"

A poll for the American Academy of General Practice found that 75% of the sample thought that their own medical care was good, but most in this poll saw "the average medical healer" as "an insincere, undedicated and somewhat unethical professional" even though a small minority agreed with the amicable comment that the "average physician" was "sincerely devoted to his work." Only 18% felt that he was completely ethical in his dealings. It is also of interest that in a discussion of a poll which indicated that doctors' prestige was second only to that of Supreme Court Justices, Gross commented that the profession of healing was too honored, too filled with magic and awe of creation and oblivion, for it to be denigrated *in spite of its present practitioners.*

There have been increasing discussions not only of the quality of patient care but its unavailability. We are all familiar with the various legislative proposals intended to make medical care available as a right rather than a privilege. George Bernard Shaw's critique of the medical profession in the preface to *The Doctor's Dilemma* indicates that the profession has a doubtful character, is infamous, deeply mistrusted, and that there is a delusion that the doctor is a man of science. However, he grudgingly grants certain virtues to the physician.

> But the true doctor is inspired by a hatred of ill-health, and a divine impatience of any waste of vital forces. Unless a man is led to medicine or surgery through a very exceptional technical aptitude, or because doctoring is a family tradition, or because he regards it unintelligently as a lucrative and gentlemanly profession, his motives in choosing the career of a healer are clearly generous. However actual practice may disillusion and corrupt him, his selection in the first instance is not a selection of a base character.

Historical Considerations

Sigerist's discussion of primitive and archaic medicine makes a number of points relevant to our theme, namely, that man has always reacted against disease and endeavored to restore health with magic and prayer, by using certain foods and drugs, and by employing methods such as healing, cooling, rubbing parts, cutting with a knife, and burning with a cautery.

Since medical theories have always represented an aspect of a general civilization of a particular period, in order to understand them we must be familiar with other manifestations of that civilization such as philosophy and literature. The healer at different times and in different cultures might be a sorcerer, a priest, a craftsman, a cleric, or a scientifically trained social worker. Since at all times society needed the physician and therefore permitted him to possess power to carry out his profession, there was a need to set up certain precautionary and protective powers against his misuse of his power.

Systems of medical ethics have developed into law to protect the public but really to protect the doctor. A series of idealized concepts for professional groups, adopted as canons of the legal and medical professions, such as the Hippocratic Oath, are in most instances a series of protective devices for the public and were brought about by distrust of the professions, especially of the healing profession.

An estimate of the ambivalence towards medicine may be arrived at, over periods of time, by the study of the evolution of the licensing and regulating procedures relating to the various "healing disciplines."

Perhaps the classic example is the so-called "Hippocratic Oath": "I swear by Apollo the physician, by Aesculapius, Hygeia, and Panacea, and I take to witness all the gods, all the goddesses, to keep according to my ability and my judgment the following Oath . . ." This invocation points up a significant aspect of the healing professions in many of the ancient cultures (Greek, Roman, Egyptian)—the original healers had the status of deities whether by birth or later deification. In all variations of the Hippocratic Oath there was a combination of the physician's basic relationship to the teacher coupled with pledges of professional activities for the benefit of the patient.

Various forms of magic were related to illness and death. In Mediterranean countries one such was the evil eye. Another widespread belief has always been the role of demons in the etiology of illness. All spirits may cause illness when they are provoked but there are differentiations between good and evil spirits. Some evil spirits have a specific function to cause illness. The ghosts of the dead, particularly those who died most recently were regarded as especially dangerous. In some cultures it was believed that only those individuals who caused disease could cure it. Perhaps one of the most unique and important documents which epitomizes the significance attributed to witchcraft is the *Malleus Maleficarum*, first published at the end of the fifteenth century, as the charter for dealing with witches. The book places a good deal of emphasis on various difficulties in the sexual sphere, supposedly caused by witches and devils. The relationships to impotence and frigidity are described almost clinically.

There were various ways in which one could become a healer or medicine man in the old cultures, but all involved an initiatory period or apprenticeship during which time the candidate would learn from the current healer the ways of the spirits of the dead, the water, the mountains, and whatever else the culture believed affected health and illness. This instruction period gave secret knowledge to the aspirant and, the knowledge made its owner a powerful and pivotal member of society.

The Egyptians, like most early cultures, attributed the control of health to the gods. Of interest is the god Set who seemed to be a special type of malignant spirit who spread and also healed certain diseases. His duality indicated "the good and the bad in the Healer"—that the one who causes disease might also cure it and vice versa. This made for an ideal ambivalent object, for the lines were blurred between the deities and their intermediaries—the medicine men.

Just as the healers were closely identified with the perpetrators of ills, they were also inseparable from the religious elements of their cultures. The distinctions between the secular and the religious were vague. These seemed to be a gradual evolution over time from priests to physicians to physicians as non-priests, even though they bore priestly titles and prestige for many years. Hermes' dual role as healer and conductor of souls into Hades makes an ideal ambivalent figure; and the Egyptian god Imhotep was both priest and physician and later deified.

Aristophanes expressed some attitudes toward physicians, namely, that they had the reputation of running after money as the doctor would not come if there was no money. From Aristophanes we learn that "It was difficult . . . to find the right doctor, and it was well known that physicians, presumably those in private practice, demanded high fees." "'Apollo the physician may cure them, since he's paid for it.'" Evidently identification with the gods and priesthood did not imply freedom from cupidity.

Aesculapius, Greek god of medicine, was born of the god Apollo and a mortal woman. This "divine man" was known as a savior of the sick and a raiser of the dead. He was a protector of the family and a predicter of the future. In some of the testimonies gathered by the Edelsteins the deeds attributed to Christ, "The Divine Physician," and Aesculapius are almost identical.

As a raiser of the dead, the god Hades complained to Zeus that Aesculapius was interfering with Hades' prerogatives in saving many from the Netherworld. It is of interest in relation to our theme that Aesculapius was ostensibly killed by Zeus because he had taken over the prerogatives of the gods, had undone some of their divine decrees as in the case of Hippolytus who had been killed by Aphrodite but was restored to life as a favor to Artemis. However, in addition to that there was an accusation that "enticed by gold, Aesculapius is said to have revived Hippolytus after he had died. . . ." so that the god of medicine too, bore the accusation leveled at healers (physicians) throughout recorded history that they loved money.

Medicine was little regarded in ancient Rome, although the Romans venerated a number of dieties who represented various medical specialties. For instance, Fever had three temples in Rome, the goddess Febris addressed as *Febris diva*, *Febris sancta* and *Febris magna*. Uterina guarded the womb. Lucina, assisted by a whole group of goddesses had charge of childbirth and *Dea Salus* was the deity who took over public health. She was the daughter of Aesculapius.

Cato was critical of medicine and lauded ancient Rome because "it was healthy without doctors." Pliny was a scorner of medical sciences and the starveling Greeks who practiced it. He maintained that "medicine, in spite of its lucrativeness, is the one art of Greeks that the serious Roman has so far refused to cultivate. Few of our fellow-citizens have been willing even to touch it, and if they do so they desert at once to the Greeks. . . . Unfortunately there is no law to punish ignorant physicians, and capital punishment is never inflicted on them."

Durant, discussing the growth of knowledge from 1300 to 1517, stated that this period was still part of the tradition called the Middle Ages in the life of Europe, dating from Constantine to Columbus, 325–1492 A.D.

> Rational studies had to fight for soil and air in a jungle of superstition, intolerance and fear. Amid famines, plagues and wars. In the chaos of a fugitive or divided papacy, men and women sought in occult forces some explanation for the unintelligible miseries of mankind, some magical power that controlled events, some mystical escape from a harsh reality; and the life of reason moved precariously in a milieu of sorcery, witchcraft, necromancy, palmistry, phrenology, numerology, divination, portents, prophasies, dream interpretation, fateful stellar conjunctions, chemical transmutations, miraculous cures and occult powers in animals, minerals and plants. All these marvels remain deathless with us today and one or another wins from almost everyone of us some open or secret allegiance.

Shryock is among the many medical historians who have written critically about the profession. He stated that the cynical distrust of the medical profession which was common in all modern centuries, seemed to go beyond ordinary bounds in Victorian decades. This showed itself in many ways, including criticism from the physicians themselves. In the eighteenth and nineteenth centuries a relatively new form of quackery grew up, namely, the patent medicines. This was also a period in which various forms of religious healing grew significantly. Religion again became a means of healing rather than an end unto itself. All the medical sects and health cults held in common a great disdain for regular medical practice and all agreed that such practice was "futile and even dangerous." Standards of medical education which were low enough declined further and private "medical colleges" abounded. Violent attacks were made by the press against the medical profession, particularly the students.

In 1858 one editor claimed that the young medics were "crude, coarse, and ignorant" and "that they went forth after an inadequate training, to contribute to the 'poisoning and surgical butchery [which] have already been committed in thousands of instances.' "

Among other criticisms in the Victorian press is the statement that there seemed to be as much quackery within the profession as without and "it has become fashionable," wrote an American medical editor in 1857, "to speak of the Medical Profession as a body of jealous, quarrelsome men, whose chief delight is the annoyance and ridicule of each other."

Nevertheless, there seemed to be some degree of popular confidence in medicine, and between 1790 and 1850 medical societies were set up that "adopted codes of 'professional ethics'" which in English-speaking countries were usually based upon the one published by Thomas Percival of Manchester in 1803. "This code stated clearly the rules of professional etiquette, and at the same time reviewed the broader obligations of physicians to society."

> There were, too, some expressions of public confidence in medicine, which appeared by way of counter-propaganda to the campaign of disapproval. "As an intellectual and thus a very useful class of the community," observed the editor of a Boston religious journal in 1831, "the medical men are entitled to high respect and esteem—they are invaluable, generous, likeable, and disinterested. They are . . . eminently entitled to the confidence of their fellow men." While the emphasis here smacks of admonition and suggests the reproval of philistines, it at least indicates the feeling that attacks on the medical profession had gone too far.

The allegation of the love of money on the part of the healer crops up again and again and is an issue to this day. Fees and payments for services of the healer seem to be a highly charged golden thread that runs through the history of all recorded cultures. John Arderne who published *Treatises of Fistula in Ano* and was a famous physician in the fourteenth century gave some advice in regard to the question of fees stating "if he does take up a case, 'let him make terms for his work and take the money in advance.'"

> He should be careful not to pronounce upon a case until he has seen it and observed what it is. When he has made an examination, even though he may think that the patient can be cured, in his prognosis he should warn him of the danger of deferring treatment. And if he sees that the patient is eager for the cure, let him boldly adjust his fee to the man's position in life. But let him never ask too little: for this is bad for both the market and the patient. . . .

Jones in his introduction to his translation of Hippocrates states that the fundamental principle of Hippocratic medicine is that nature alone can effect cure and that the physician should allow nature a chance to work. "Nature," says he, or at least one of his immediate followers says, "is the physician of diseases. . . ." A general rule still quoted of Hippocrates was "to do good, or at least to do no harm." According to Plato, Hippocrates was an "Aesculapiad"—a descendant of Aesculapius and thus related to the god of medicine.

> The physician must be able to tell the antecedents, know the present, and foretell the future—must meditate these things, and have two special objects in view with regard to diseases—namely, to do good or to do no harm. The art consists in three things—the disease, the patient, and the physician. The physician is the servant of the art, and the patient must combat the disease along with the physician.

Hippocrates' discussion on the Sacred Disease within the context of his time and the climate relating to causes of disease is of prime significance since it tells us a good deal about the then current non-naturalistic theories of etiology:

> And this notion of its divinity is kept up by their inability to comprehend it, and the simplicity of the mode by which it is cured, for men are freed from it by purifications and incantations.
>
> And they who first referred this disease to the gods, appear to me to have been just such persons

> as the conjurors, purificators, mountebanks, and charlatans now are, who give themselves out for being excessively religious, and as knowing more than other people. Such persons, then, using the divinity as a pretext and screen of their own inability to afford any assistance, have given out that the disease is sacred, adding suitable reasons for this opinion, they have instituted a mode of treatment which is safe for themselves, namely, by applying purifications and incantations, and enforcing abstinence from baths and many articles of food which are unwholesome to men in diseases.

And again there is advice in the Hippocratic Corpus relating to the question of fees:

> I urge you not to be too unkind, but to consider carefully your patient's superabundance or means. Sometimes give your services for nothing, calling to mind a previous benefaction or present satisfaction. And if there be an opportunity of serving one who is a stranger in financial straits, give full assistance to all such. For where there is love of man, there is also love of the art. For some patients, though conscious that their condition is perilous, recover their health simply through their contentment with the goodness of the physician. And it is well to superintend the sick to make them well, but also to care for one's own self, so as to observe what is seemly.

Closely related to the subject of the physician is the serpent, the symbol and sign of the healer throughout history in most cultures, oriental and western. It has represented life, death and rebirth in all its various forms. Tempter, Devil, Satan, and Evil One are other roles attributed to and played by the Serpent. The duality of its symbolism parallels the duality of the physician's role and the ambivalence manifested toward both the serpent and physician. Healing and death, good and evil, male and female, and creation and destruction are but a few of the roles the Serpent has been cast in. It appears on the caduceus; and it is a protagonist often in the Bible. Chapter 3 of Genesis presents the matrix for sorrow and disease as punishment for eating the fruit of the Tree of Knowledge at the instigation of the serpent. It is of significance that in order to keep mankind from immortality man is driven from the Garden of Eden and prevented from returning to eat of the fruits of the Tree of Life. God's power to perform miracles and create sickness is again demonstrated in the Bible in Exodus 4 when at Horeb Moses expressed his unwillingness to carry God's message to the Jews concerning their proposed exodus, stating that they would not believe that he had a message from God. He was ordered to throw his rod on the ground where it became a serpent that frightened Moses so that he fled "before it." He was instructed to take the serpent by the tail and it again became a rod. Still unwilling, he was told to put his hand in his bosom and it became leprous. When he again put it in his bosom "behold, it was turned again into his *flesh*." He who controlled the serpent did so against a background of good and evil.

Innumerable passages in the Old Testament firmly establish illness in its various forms as punishment from God for disobedience of his commands, for lack of piety, and the worship of other gods. All of this had a direct relationship over the ensuing centuries to the practice of healing.

The New Testament on the other hand emphasizes over and over again the healing role of Jesus. In St. Luke 6, verses 6 to 10, Jesus healed the man with the withered right arm on the Sabbath. In St. Luke 7 he raised the young man from the dead since he had compassion for the mother.

The contrast between the Old and the New Testament in relation to illness, death, and healing lies in the Old Testament's emphasis on illness as punishment and the New Testament's emphasis on healing as reward for belief. Each one of these attitudes resulted in a kind of vector in the subsequent development of Judaism and Christianity.

Dr. Immanuel Jakobovits, currently the chief rabbi of Great Britain has written an interesting volume on Jewish medical ethics. He draws attention to the essential prophylactic nature of Jewish medicine. He points out that in the relationship of human healing vs. divine providence—there are essentially three attitudes towards illness: one was that the sick man is one who is burdened with a certain odium; in ancient Greece illness was a curse; and in the Christian religion, which is the religion of healing, there is the atoning power of illness. The conflict between providential design and interference by the physician is resolved by three completely divergent approaches: one might reject medical aid; it was the duty of man to harness his intelligence for the conquest of disease; or God granted man the right to cure and the physician practiced under this sanction.

Over the years Talmudic and other authoritative interpretations held that the Bible explicitly warranted the employment of physicians and medical cures. The Christian schoolmen in the Middle Ages cited Ecclesiasticus 38, verse 4 (Sirach) as divine sanction for human healing. The aspects of the controversy were phrased in various ways such as: "he who heals might appear as if he invalidated a divine decree" or "permission is required 'lest it be said that God smites and man heals. . . .'"

"Jewish religious leaders in all ages have consistently urged those threatened by pestilence to put to flight without hesitation." This is in spite of the fact that "prayer and faith" were often used as the "sole therapeutic agents in the fight against disease."

There were many superstitious, occult, and scatalogical cures. Prayer and exorcism were considered effective, particularly among the Christians. The role of witches was especially prominent. St. Augustine is quoted as declaring that: "All diseases of Christians are to be ascribed to demons. . . ."

Even though various superstitions, sorcery and witchcraft were severely condemned by the Jews the sorcerer's powers were never questioned.

The right to practice and the legal responsibilities of the physician are traced back as early as the second century A.D. It was in the middle of the twelfth century that the Norman King Roger introduced licensure and ". . . in Paris at the beginning of the 16th century" licenses "were" sometimes "sold for money" therefore, "some students refused to take any degree. . . ."

A report of conditions in England at the same time declares that "the practice of physic was improperly supervised, and had fallen into the hands of smiths, weavers and women. Midwives were licensed by the same authorities." These abuses led to the parliamentary statute of 1511 providing that "no person within the city of London . . . take upon him to . . . occupy as a physician or surgeon, except he be first examined, approved and admitted by the Bishop of London . . . sided by four doctors of physic, or persons expert in surgery. . . ." In 1540 the statute was followed by an Act which empowered the United Company of Barber Surgeons to impose fines on unlicensed practitioners in London. Medical degrees were also granted by the popes and papal legates.

In 1310 the synod of Traves decreed: "Inasmuch as so many ignorant physicians have arisen, no-one shall hence-forth practice or teach medicine and surgery without the permission of the bishop who shall examine the applicant both in respect to his knowledge and his morals."

It is also of interest that in both ancient and medieval times there were laws relating to the physician's liability for damages—the Code of Hammurabi was essentially an aspect of *lex talionis* in which a doctor was liable to have his right arm removed if an operation he performed was fatal. A "Persian law punished a surgeon whose patient died for 'wilful murder,' unless he had previously performed successful operation on three unbelievers. In Egypt a physician could be put to death if he treated anyone in an unauthorized manner with fatal consequences."

In both Jewish law and practice the physician's right to payment for services was recognized. Apparently wide currency was enjoyed by the statement that "a doctor for nothing is worth nothing" and Isaac Israel's aphorism of the 10th century stated: "The more thou demandest for thy service . . ., the greater will [it] appear in the eyes of the people. Thine art will be looked upon as insignificant by those whom thou treatest for nothing."

In Jewish law, a discussion of the priest as physician draws attention to the ban on priests as physicians which is related to defilement and the fear of the dead. This ban was present in Christianity but for different reasons. Christians considered the two callings as fundamentally incompatible. In Judaism it was so because of the laws of priestly defilement.

In Christian doctrine it is exemplified by a decree in which the Council of Tours in 1163 held that:

> the devil, to seduce the priesthood from the duties of the altar, involved them in mundane occupations which, under the plea of humanity, exposed them to constant and perilous temptations.

In *The Apocrypha* there is a section entitled "The Wisdom of Sirach," which appears as Ecclesiasticus 38 in the Catholic Bible, states that the physician should be honored because of one's need for him and since the Lord had created him. The ultimate relationship between the physician and the Lord in relation to illness is stated thus:

> Show the physician due honor in view of your need of him,
> For the Lord has created him;
> Healing comes from the Most High,
> And he will receive presents from the king.
> The skill of the physician exalts him,
> And he is admired among the great.
> The Lord has created medicines out of the earth. . . .

Throughout the Judeo-Christian era to this day, there has been a recurring question as to the right of a human healer to treat and cure which might be looked upon as invading God's prerogatives to cause disease and death. This during the Greek era might be designated as the crime of Aesculapius.

The current concern in relation to malpractice is part of a historical development. An excellent example is Cosman's recently published article on medieval medical malpractice in which she discusses seven major malpractice cases dating back to 1354 with the names of the plaintiffs and defendants and with the following charges and dispositions:

> "Maiming" and neglect of jaw wound
> Verdict: Guilty
>
> "Endangering" leg
> Verdict: Guilty
>
> Deceit and falsehood in treating woman
> Verdict: Guilty
>
> Failure to cure and excessive fees
> Verdict: Guilty
>
> "Iatrogenic sequelae," maimed hand
> Verdict: Not guilty

This would indicate that patients had recourse for "bad medicine."

Arderne in the 15th century provides us with a description of how the physician should behave. He urged what might be called a low profile. The physician should not look too boldly at the lady of the house, her daughters, or "other fair women in great men's houses, or offer to kiss them, or touch them with his hands, lest he move to indignation the lord or some one of his household." He should not be too haughty or too familiar with sick men and their friends "but adapt his manner to the status of the persons: to some respectful, to others friendly." He should be well dressed and neat in appearance "not gay like a minstrel but sober like a clerk." He then may sit at a gentleman's table. He should listen well and say little. A doctor should not be rash or boastful in speech or deed; he should answer all questions cannily to avoid being tripped up, should not laugh and joke too much, should avoid the company of knaves and dishonest persons, should be busy with matters pertaining to his profession—reading, writing or praying, should be sober, should say about other doctors, "I have no real knowledge of him, but I have heard nothing about him but what is good and to his credit. . . ."

The annual academic address to the Massachusetts Medical Society by William Williamson Wellington in 1870 again illustrates the long history of ambivalence to the doctor. "It must be confessed that the manner in which the medical profession is regarded by the community at large is not such as in all respects to gratify our self-love, or to flatter our vanity."

William H. Prescott, the historian, is quoted from a letter in which he expresses his sentiments towards medicine:

> I am delighted that you are turning a cold shoulder to Aesculapius, Galen, and tutti quanti. I detest the whole brotherhood. I have always observed that the longer a man remains in their hands, and the more of their cursed stuff he takes, the worse plight he is in. They are the bills I most grudge paying, except the bill of mortality, which is very often indeed sent in at the same time.

Another criticism in the Wellington paper which has a strong current flavor is the following:

> It is to be regretted that the pressure upon medical men to polypharmacy is so strong, that he is a brave man who can resist it. Many persons seem to believe that for every disease, and for every symptom, there is, either in the earth or on it, a remedy in the form of a drug; and if the patient does not get this remedy, that the fault lies with the doctor. In fact, the only idea some people have of a physician is that he is a man who gives medicine; should he make a medical visit and not give medicine, they would think he has mistaken his calling, and would probably seek other advice.

An additional criticism of medicine as practiced in the 1870's is as follows and might be called the Lament of the Lost General Practitioner:

> A marked feature in the profession of to-day is the tendency to carry the subdivisions of medical work to an extreme degree. Formerly the only division considered necessary was between medicine and surgery, and that only in the larger cities. Soon dentistry became a specialty, and properly so . . . We may well acknowledge our indebtedness for some of the most brilliant discoveries of the century to individuals who have given especial devotion to some one branch of their profession; but we must not forget that they have been men whose general acquirements were not limited to the subject they have illustrated, but that these were the solid foundation on which, alone, they were enabled to push their special investigations to such brilliant results.

Death customs and burial rites are usually indices in any particular culture of attitudes towards the living and the recent dead, at both the conscious and unconscious levels. The attitudes towards the healer as medicine man or shaman are of interest, since some believe that "only the medicine man was able to cause disease and therefore when a death occurred it had to be traced to a definite individual medicine man who was responsible for the death," and in certain tribes under these circumstances "he had to fight against the relatives of the deceased." In North Australia for instance, with the aid of the medicine man, it was possible to discover the real cause of death; and it was the medicine man's role to organize an avenging party to kill the guilty person.

The problem as to why the dead were supposed to radiate such danger especially to those to whom the dead had been attached and dearest in life has been a recurring problem to many ethnologists. The taboo aspect of the corpse manifested itself in many variations in practically every culture even to this day. There is an uncanny aspect to the corpse with power for evil in most instances in direct proportion to the rank and sanctity of the deceased. The Jewish priest cannot be in the presence of the dead body [Leviticus XXV, verse 2] and therefore a member of the priestly tribe [Cohen] is traditionally not permitted to become a physician.

Freud in his *Totem and Taboo* leans heavily on Frazer and makes references to the appeasement by warriors through sacrifices offered to the souls of the men whose heads had to be taken in warfare. Frazer discusses the taboo upon the dead and emphasizes the dire consequences that follow contact with the dead. Among the Maoris anyone who handles the dead or takes part in the burial is unclean—is boycotted and cannot touch food. (This may be currently reflected in the cultural attitudes toward morticians.) Of interest also in this connection is Freud's 1919 article on "The Uncanny" in which he says:

"Many people experience the feeling in the highest degree in relation to death and dead bodies, to the return of the dead, and to spirits and ghosts."

Freud, in his *Thoughts for the Times on War and Death* (1915) formulated one aspect of the problem in this way:

> We ask what is the attitude of our unconscious towards the problem of death. The answer must be: Almost exactly the same as primitive man's. In this respect, as in many others, the man of prehistoric ages survives unchanged in our unconscious. Thus, our unconscious does not believe in its own death; it behaves as if immortal.

Feder has stated as a result of his psychiatric work with patients undergoing radiotherapy:

> It has been my experience that practically all patients who have a malignant disease "know" in some way that they have it . . . All these patients, when they were asked to see me as a "new doctor," reacted with great anxiety. Of the first 50 patients, the only one who showed no anxiety was a woman in her fifties who was overtly psychotic in every respect and who reacted with no apparent anxiety to anything. All the other patients were terrified at going to see a new doctor. They wondered: "What has gone wrong? . . . This is it . . . Now I am going to hear *it*." These were the same patients who had convinced the radiotherapy staff that they accepted the diagnosis of "inflammation," "tumor," or "arthritis."

Of some relevant interest in relation to the confrontation with death would be the two statements of Christ on the Cross who at first says (St. Luke 23:34): "Father, forgive them, for they know not what they do . . ." but in the end utters the most poignant cry that has come down through history (Matthew 28:46):

> And about the ninth hour Jesus cried with a loud voice, saying, E-li, E-li, la-ma sa-bach-tha-ni? My God, my God, why hast thou forsaken me?

Throughout many cultures the ruler or sovereign was a combination of priest, healer, and ruler and Freud quotes Frazer that a ruler "must not only be guarded, he must also be guarded against."

The healing power of the royal touch in regard to the King's Evil was a phenomenon that lasted into the 17th century and the laying on of hands is still part and parcel of current belief. The role of the King as the protector not only of health and life but the totality of the functions of nature had its obverse side. Failure to function in this role made him open to hostilities leading even to his death from his disenchanted subjects. Hostilities, aggression, and denigration of the physician and the medical profession in the situation where his magic fails is thus easily understood. One can readily extrapolate the taboo ceremonials discussed by Freud as they would relate to the healer:

> . . . our starting-point must be the excessive apprehensiveness and solicitude which is put forward as the reason for the taboo ceremonials. The occurrence of excessive solicitude of this kind is very common in which our comparison is chiefly drawn. We have come to understand its origin quite clearly. It appears wherever, in addition to a predominant feeling of affection, there is also a contrary, but unconscious, current of hostility—a state of affairs which represents a typical instance of ambivalent emotional attitude. The hostility is then shouted down, as it were, by an excessive intensification of the affection, which is expressed as solicitude and becomes compulsive, because it might otherwise be inadequate to perform its task of keeping the

> unconscious contrary current of feeling under repression. Every psychoanalyst knows from experience with what certainty this explanation of solicitous overaffection is found to apply even in the most unlikely circumstances—in cases, for instance, of attachments between a mother and child or between a devoted married couple. If we now apply this to the case of privileged persons, we shall realize that alongside of the veneration, and indeed idolization, felt towards them, there is in the unconscious an opposing current of intense hostility, that, in fact, as we expected, we are faced by a situation of emotional ambivalence.

An interesting comment concerning the above is the finding that many of the polls and studies about physicians indicate that in spite of the hostility and devaluation of the medical profession there is an almost universal exception made in relation to one's own physician.

Psychological Considerations

What light can psychiatry and psychoanalysis throw on the foregoing evidence of such widespread ambivalence, toward the healers throughout recorded history?

In specific terms, a patient's reaction to illness is an intrinsic part of the doctor-patient relationship and consequently a significant factor in determining the patient's attitude to the physician. Some of the coping techniques used by patients to face illness, as outlined by Lipkowski, include minimization, denial, capitulation, regarding illness as either an enemy, a challenge or a punishment.

Patients' attitudes toward the physician are directly related to the Oedipus situation. In mythology gods and goddesses of legend would seem to be direct projections of the parental figures. Mother as the first healer to the child is a ubiquitous phenomenon and we are all familiar with the fact that "when it hurts, Mother's kiss makes it better."

In the light of the role of the healer some discussion of the factors that make for the choice of medicine as a profession are of interest, especially since historically the various roads to healership have been determined by heredity, self-choice, a call or sense of mission, or because of survival through witchcraft and a way of dealing with demons.

Several psychoanalysts have written about the unconscious factors that may determine the selection of medicine as a profession. Nunberg emphasized the role of doctor games in childhood following an illness affecting the child himself or some person in his environment. There is a need to repeat the traumatic experience of the illness again and again: the compulsive repetition serves in some way to master the traumatic experiences. In many of these games there is an alternation between playing the role of the doctor and that of the patient. Nunberg postulates that following this the physician may identify with the patient in relation to various procedures and unconsciously see illness as due to magic and healing as countermagic. In the light of these unconscious sources that lead to the selection of medicine as a profession, one might profitably take a look at the medical student and try to evaluate the entire process of education for medicine.

The patient-physician relationship, based as it is on reciprocal emotional attitudes, is a very important element in the art of medicine. There are multiple factors which enter into this relationship. It is often not realized that the physician plays a psychological role which is not altogether determined by reality. This is evidenced by the devotion of many patients to the family practitioner, not because he is a better scientific physician, but because he is a combination of father confessor, authority, and arbiter in the solution of family difficulties.

The physician, as a rule, is accustomed to expect only favorable reactions and tends to accept himself at the patient's overevaluation. The emotional attitudes of the patient to the physician are based upon earlier experiences. The physician may be identified with figures, such as the father, who played an important role in the early life of the patient. In addition the role of the physician is determined by many other factors, not the least of which is the expectation of a cure. As a rule the positive aspects come to the forefront rather early. Sooner or later, the patient, either because of disappointment, frustration, or identification of the physician with earlier figures, may acquire certain hostile reactions. The physician on his part may also develop certain emotional reactions towards the patient. "Good" patients are those whom they can cure easily, and "bad" patients, those they cannot cure. Or the attitude of the physician may depend upon the type of illness presented by the patient: whether the illness is understood in terms of organic etiology, or is to some degree of neurotic origin and therefore not evaluated properly.

Organic disease has an extrinsic significance to the individual who is ill. This is aptly demonstrated in the case of syndromes which might be termed "stigmata illnesses." An individual who becomes infected with syphilis, gonorrhea, or tuberculosis—diseases which are surrounded by certain cultural taboos—will react in terms of his previous attitude to the taboos. The luetic after learning of the diagnosis, may avoid his physician, and rather than continue treatment, may be driven to a suicidal attempt because of his sense of guilt. It is the experience of many syphilologists that an individual may terminate his treatment as soon as the serologic tests become negative, not because he is cured, but because the patient believes he is cured, in order to avoid the guilt associated with the illness. On the other hand, the patient may not believe the physician when told he is cured. His anxiety may be so great that he needs constant reassurance, returning again and again for further tests and treatment. Certain organic illnesses which are associated with fear of death, such as cancer, may lead to a psychological denial of the seriousness of the situation. One may observe such a mortally ill person, apparently full of optimism, constantly making plans for the future.

If the diseased organ happens to be one that has been used in the solution of certain conflict situations, the subsequent reaction to the illness may be of considerable significance. Freud refers in his writing to the need to be ill from a sense of guilt emphasizing the role of the superego. The hypochondriac, who because of guilt and anxiety has always feared heart disease, may welcome actual heart involvement as a punishment for his unconscious fantasies; or his anxiety may increase to tremendous proportions.

In addition to the writings of Schilder and Kolb, Weinstein and Kahn deal with denial of illness in its symbolic and physiologic aspects. The literature of denial of illness concerns itself for the most part with cases of blindness and hemiplegia. Babinski in 1914 first reported anosognosia in a patient with left hemiplegia. The term anosognosia means "lack of knowledge of existence of disease" and has been noted in patients with right hemiplegia, paraplegia, absence of amputated limbs, deafness, alexia, auditory agnosia, with the denial of having undergone operative surgery, and the denial of other types of illness. Head and Holmes in 1911 and Pick in 1908 considered anosognosia as a disturbance of "body scheme." There has been a good deal of discussion as to whether an organic basis is necessary. Schilder spoke of "organic repression." Patients excluded the unpleasant facts of hemiplegia or blindness from consciousness. It is of interest in relation to our theme that the relatives of Babinski's patients considered this denial as "delusion prudential" and they asked the doctors to avoid all questions that might "disenchant them [the patients] and trouble them in their peace."

Goldstein (1939) considered denial as a psychological mechanism present in the normal individual and that this was a type of adaptation. Sandifer (1946) took into account the motivation of the patient to be well, thus avoiding "catastrophic reaction."

Anna Freud, in her discussion of the ego's defensive operations, refers specifically to the relationship of the patient to the analyst. One may extrapolate "physician" in the analyst-patient relationship for much of what she states. The physician then becomes the "menace" in the quote: "hence except insofar as the patient's insight into his illness determines the matters otherwise, the ego-institutions regard the analyst's purpose as a menace."

Antagonism to the fact of illness rejects other certain facts and prevents them from coming to light. Hostility to the physician develops—a negative transference type of reaction is involved. There is a defense against the affect which may appear with the recognition of the dangerous situation, i.e., illness.

The ego has only a limited number of defensive maneuvers available—repression, displacement, reversal, suppression of emotions, and denial.

The technique of defense against the painful affects are like the defenses against primarily painful situations which impinge from the outside world. This stimulates hostility against the bearer of bad news, the physician, and may be denied in fantasy and in word and deed.

The individual variations of the universal pre-Oedipal and Oedipal situations that are laid down as prototypes relatively early in life then become the significant behavior patterns for the future, since, as already indicated the physician's role makes for many transference epiphanies that lead to a series of ambivalent relationships at the conscious, preconscious and unconscious levels.

It is essential to take into consideration that there are many "reality factors" that enter into the matter at any particular time and that these must be given due weight in any formulations.

The actual defects in the delivery of medical care that occur at any given time may be the analogue to the "fragment of historical truth" in delusions. This fragment may be a very large one.

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OPHTHALMOLOGIC NOTES

KEITH M. ZINN, M.D., EDITOR

Congenital Pits of the Optic Nervehead and Associated Serous Detachments of the Macula*

Congenital pits of the optic nervehead were first described in 1882 by Wiethe (1) as crater-like depressions on the surface of the optic disc. These optic pits vary in their location, size, depth, and overall configuration (circular or oval). The diameter of the pits ranges from ⅓ to ⅙ of a disc diameter and their depth ranges from two to seven diopters (0.7 to 2.7 mm) (2, 3). In most cases, there is only one pit in a disc and the condition is unilateral. However, multiple pits in a disc as well as bilateral cases of optic pits have been reported (4). The pits are usually grey or greyish-white and, according to Kranenburg, 40% are touching or nearly touching the disc edge whereas 36% of cases are between the center and the disc edge and only 4% are located centrally (5) (Table I).

The incidence of congenital pits of the optic nerve has been estimated by Reis (6) and Kranenburg (5) to be 1:11,000 patients with ocular complaints whereas Gjessing's estimate was 1:62,300 (7). The age of incidence varies from infancy to 73 years, averaging about 35 years. Men and women are affected equally. However, Kranenburg (5) found 57% of reported cases involved the left eye and 43% involved the right eye. Congenital pits have also been reported in association with optic nerve colobomas (4, 5, 8), serous detachments of the macula (2, 5, 9–13), and other macular abnormalities, e.g., macular edema, macular holes, macular hemorrhages, and macular cysts (14). Gass (9) has pointed out that enlarged optic nerveheads are frequently associated with optic pits. Visual field defects, e.g., central scotomata, enlarged blind spots, and arcuate scotomata are found in 50% of cases (5). Macular involvement occurs in 25% (5) to 32% (2) of cases, and the onset is usually between the ages of 20 and 40 years (9).

There is some debate over the actual pathogenetic mechanisms involved in the development of pits of the optic nervehead. Many authors have stated that optic pits are colobomatous lesions that are in some way related to malclosure of the fetal fissure. However, Gass (9) believes that the optic pit is basically related to abnormal development of the primordial optic nerve papilla with failure of the normal resolution process involving the peripapillary neuroectodermal folds. Histopathologic studies by Peterson (15) and Ferry (13) have demonstrated that the optic pit is filled with primitive or rudimentary retinal

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tissue with associated glial elements, pigment epithelium, and nerve fibers. Kranenburg (5) and Ferry (13) stressed the importance of damage to the maculopapillary nerve fiber bundle in the pathogenesis of serous detachments of the macula in cases with optic pits.

The source of the subretinal fluid in those patients with optic nerve pits that develop serous detachments of the macula remains controversial. However, two extravascular sources for the subretinal fluid are available. First, there is the possibility that the fluid originates in the vitreous cavity (16) entering the optic pit directly which, in turn, allows the fluid access to the subretinal space resulting in serous detachment of the macula. The major evidence against this vitreo-subretinal fistula theory is the failure, on histopathological examina-

TABLE I

Distribution of Pits on the Optic Nervehead (5)

Location of Optic Pits	% Cases
Inferotemporal	46%
Midtemporal	26%
Superotemporal	4%
Midsuperior	3%
Midnasal	4%
Nasal	3%
Inferonasal	7%

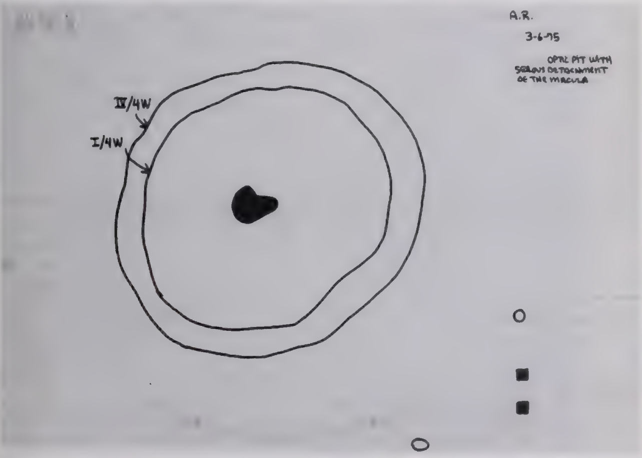

FIG. 1. Visual field showing cecocentral scotoma in an eye with an optic pit and serous detachment of the macula.

tion, to demonstrate acid mucopolysaccharides within the depth of the pit or in the subretinal space where the macular detachment is present. A second possibility, put forth by Regenbogen et al (11) and Gass (9), is that cerebrospinal fluid might gain access to the subretinal space by passing between the fibrous capsule of the pit and the scleral rim or by entering the pit capsule beneath the invaginated neuroectoderm. At the present time, neither theory has been completely proved or refuted by the clinical and histopathological evidence. A third possibility involves the leakage of fluid from retinal, choroidal, or disc vessels (17) into the subretinal macular zone. Fluorescein angiographic studies failed to demonstrate any dye leakage from these circulatory systems into the subretinal space, thereby negating the latter theory of vascular incompetence (9). However, in the case presented in this paper, there does appear to be some fluorescein dye leakage into the macula associated with the optic pit.

Photocoagulation along the temporal edge of the optic nervehead has been tried as a treatment for serous detachment of the macula in cases with pits of the optic nervehead, but with poor results (9).

The following case is presented to demonstrate a congenital optic pit with serous detachment of the macula which first presented as unilateral metamorphopsia.

Case Report

A 41-year-old pipefitter complained of distorted vision in his left eye for the past three weeks. There was no history of pain, fever, headache, or malaise associated with the progressive loss of vision in the left eye. Ocular examination was as follows:

Visual acuity, without correction, was 20/20 in the right eye, and 20/400 in the left eye. Manifest refraction, with a +1.50 sphere, brought the visual acuity up to 20/50+2 in the left eye. Motility studies were within normal limits. Cranial nerve function III, IV, ophthalmic division of V, VI, and VII were intact, bilaterally. The Amsler grid test was normal on the right and indicated metamorphopsia on the left. The visual field on the right was entirely within normal limits, whereas there was a cecocentral scotoma on the left (Fig. 1).

The lids, ocular adnexae, and lacrimal gland apparatus were entirely within normal limits, except for a small papilloma on the left lower lid margin. The conjunctivae and corneae were clear and unremarkable, bilaterally. The sclerae were within normal limits, bilaterally.

Intraocular pressures were 15 mm Hg in either eye, by applanation tonometry.

Slit-lamp examination of the anterior chambers revealed them to be clear, of moderate depth, with Grade III open anterior chamber angles, and 2+ pigmentation of the trabecular meshwork. The irides were brown, and the pupils were equal, round, and reactive to light and accommodation.

The lenses and vitreous cavities appeared to be clear and unremarkable, bilaterally.

Funduscopic examination on the right was entirely within normal limits. However, the funduscopic examination on the left revealed the disc to have an optic pit on its temporal margin with a possible secondary depression (pit) at the 3:30 to 4:00 o'clock meridians. Adjacent to the disc was a serous detachment of the macula and intraretinal exudate was also present (Fig. 2). The vein/artery ratio, at the disc margin, was 3:1.5, in both eyes.

Fluorescein angiography was performed (Figs. 3–5), and the hypofluorescent zone on the temporal disc corresponded to the optic pit. There was dilation of the superficial disc capillaries at the borders of the optic pit (Fig. 3) which began to leak in the late arteriovenous phase, staining the walls of the optic pit and its immediate borders. There was some leakage of dye from the optic pit region into the adjacent subretinal space temporal to the disc (Figs. 4, 5).

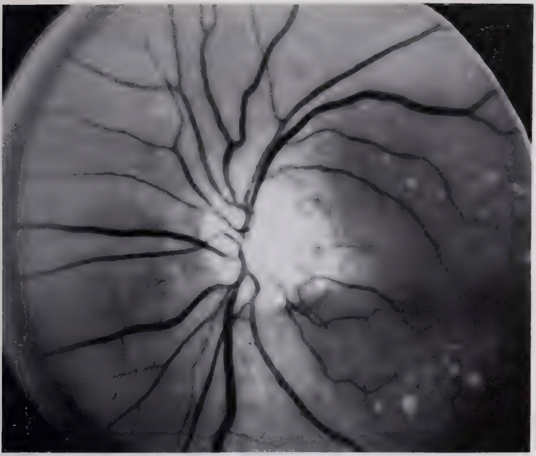

FIG. 2. Red-free photograph of disc and macula showing an optic pit on the temporal border of the disc with a possible second pit at the edge of the disc at the 4 o'clock meridian. Intraretinal or subretinal exudate is also present.

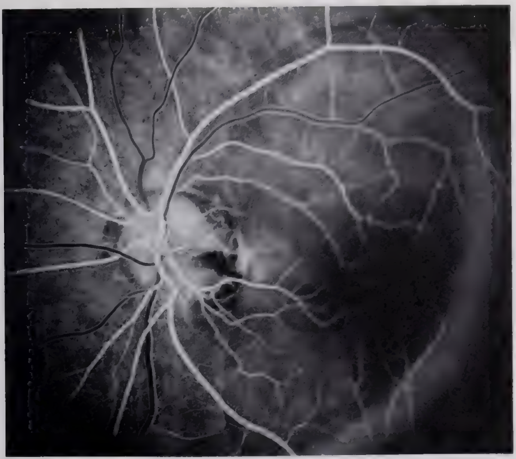

FIG. 3. Fluorescein angiogram—early arteriovenous phase. Hypofluorescent zone on temporal disc corresponds to the optic pit. There is some dilatation of disc capillaries adjacent to the optic pit.

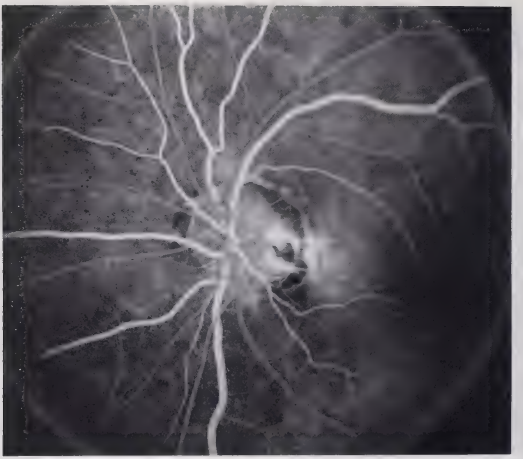

FIG. 4. Flurescein angiogram—late venous phase. Staining of capillary system adjacent to the optic pit with possible leakage into subretinal space just temporal to the disc.

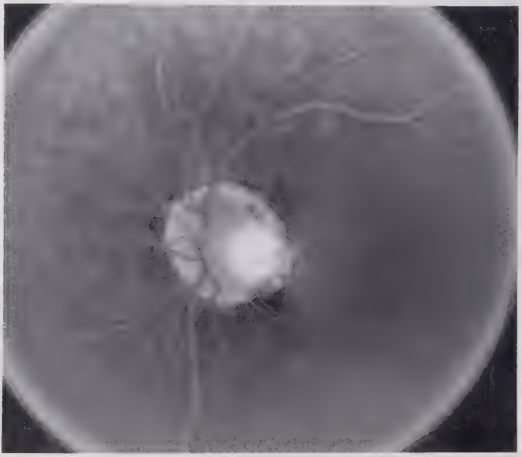

FIG. 5. Fluorescein angiogram—late shot showing hyperfluorescence and staining of optic pit and adjacent disc tissue. Little, if any, fluorescein is seen pooling in the subretinal space.

Dark adaptation tests were within normal limits on the right, whereas the left eye showed normal rod thresholds, but the cone thresholds were elevated by approximately 0.8 to 1.0 log units and the transition from the cone to the rod branch of the dark adaptation curve was delayed.

Electroretinography was performed and was entirely within normal limits on the right. Electroretinography on the left revealed normal a- and b-wave amplitudes to white, red, and blue stimuli. Although the a-wave produced by the scotopic white stimulation was borderline supernormal. The results of the electrophysiologic tests were consistent with some degree of localized retinal dysfunction, i.e., serous detachment of the macula.

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MEDICOLEGAL NOTES

MICHAEL G. MACDONALD, J.D. AND KATHRYN C. MEYER, J.D., COEDITORS

Informed Consent

Few medicolegal concepts have generated as much debate, confusion, and indignation as the doctrine known as "informed consent." Judicial decisions abound in the area; federal and state governments have imposed comprehensive regulations; and professional meetings throughout the country have made informed consent their preoccupation. During the recent malpractice insurance crisis, the debate reached a crescendo. Physicians contended that informed consent was an arbitrary legal requirement that interfered with their responsibilities toward their patients for the sole purpose of enriching the legal profession. In response to such criticisms, many states, including New York, enacted statutes which attempted to restrict the right of a patient to recover damages from his physician for lack of informed consent.

We believe that much of this controversy results from confusion as to the real purpose of informed consent. Once the rationale and purpose of the informed consent rule are understood, we submit that it can be seen not as an arbitrary or burdensome legal doctrine but as an essential part of the physician/patient relationship and of proper patient care.

The basic premise of informed consent is that every human being has a right to determine what shall be done with his or her body. The first cases on consent arose in a branch of law called assault and battery, which gives the individual the right to recover damages for a nonconsensual touching. In the oldest New York case, a patient told her physician that she was willing to undergo an examination but did not want an operation; he proceeded to anesthetize her and remove a fibroid tumor from her uterus. Later, when complications arose as a result of the surgery, she sued. The court, in a famous opinion by Justice Cardozo, declared: "Every human being of sound mind and adult years has a right to determine what shall be done with his own body and a surgeon who performs an operation without his patient's consent commits an assault for which he is liable in damages." As this consent requirement evolved over time, the courts came to recognize that the patient needed more than the simple right to say "yes" or "no"—that the right to decide meant very little if the patient had no idea what he was deciding. The physician was thus required to obtain his patient's *informed* consent.

Initially this right to receive information included only that information which *physicians* in the same general locality customarily disclosed and in some cases this meant that patients were still told little or nothing at all. This ptolemaic view of the consent process has gradually begun to erode, however, and today in most states a physician must disclose that information which a

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reasonable *patient* would consider relevant in deciding whether or not to undergo a course of treatment.

The point to be gleaned from this is that informed consent is essentially a process of communication designed to facilitate the patient's ability to make the important decisions about what will happen to him. It is thus an integral part of the physician/patient relationship. This may all seem self-evident, but it is interesting to note how far practice seems to have strayed from the basic purpose of the rule. A few examples of the more troublesome informed consent questions illustrate the point.

1. Should consent be in writing? Doctors, nurses, and others often believe that consent must be in writing in order to be legally valid. Consent is not seen as part of the process of communication, but as a yet another legal requirement that must be satisfied. Unfortunately, the attitude becomes self-fulfilling: when this approach is dominant, the entire focus is on obtaining the patient's signature. As a result, patients are often asked to sign consent forms with little or no meaningful explanation. In point of fact, with few exceptions, the law does not require consent to be in writing. Consent is a process and a "consent" has been given when a patient indicates his agreement by whatever means suits him, not when he signs his name. The requirement at most hospitals that a written consent form be obtained for more dangerous procedures is simply a documentation requirement; if questions are later raised, the form helps the physician and the hospital prove that the consent was in fact obtained. But a signed consent document is of no value if the basic communication has not taken place.

2. When must consent be obtained? As a corollary to the mistaken concept that consent must be in writing, physicians sometimes assume that consent need only be obtained for major procedures. Again, consent is the right of the patient to control what happens to him, and this right does not disappear if the treatment in question happens to be routine or without risk. The amount of information which must be given may be much less in this latter situation and the law is sometimes willing to infer a patient's consent simply from his presence in the hospital or doctor's office, but the patient never loses his right to say no. Similarly, it is common practice not to obtain patient consent for drugs unless they are experimental. But, the possible side effects and dangers from a drug may be equal to those of major surgery; the fact that the treatment is not intrusive does not eliminate the need for patient decisionmaking.

3. Must consent be witnessed? Many consent forms provide for the signature of a witness, and the conclusion is sometimes drawn from this that without such a witness the consent is not legally valid. But, if consent is a process of communication between the physician and patient, the presence of a third person is clearly not legally necessary.* Witnesses, like written consent forms,

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* In the areas of human experimentation and sterilization, special federal regulations require the presence of an "auditor-witness" during the informed consent process. The regulators clearly felt that in these two areas, the possibility of manipulation of the subject/patient was sufficiently great to require the presence of a third person.

serve a documentation function. In the event of a later dispute between the physician and the patient, they are available to testify as to whether the communication in fact took place. But a consent that is not witnessed is a perfectly good consent.

4. Who should obtain the consent? It is frequently the practice, particularly in large hospitals, to rely upon the nurse to obtain the patient's consent. The answer here is obvious: if consent is physician/patient communication, the physician should be the one to make the communication. Similarly, if a consent form does require the signature of a witness, clearly the first choice for the role should be the physician, since he is the person making the communication.

5. If consent forms are used, must they contain a long description of every possible risk and complication? Physicians sometimes believe that they can protect themselves from liability by presenting their patients with an elaborate form detailing every possible piece of relevant information. But, the physician's responsibility is to communicate with his patient and he cannot discharge that duty by relying on written forms. A short consent form may in fact be preferable, since it encourages the physician to make the disclosure himself directly to the patient. The short form can be accompanied by an outline which helps the physician remember the points he wishes to cover, insures consistency among patients and provides evidence as to the content of the conversation in the event of later questions.

The final point we wish to make about informed consent is that efforts to restrict the informed consent rule by legislation may have the unanticipated result of increasing the exposure of physicians to malpractice suits. It is generally agreed that the increase in malpractice claims is at least partially a response to certain dehumanizing trends in modern medicine. Medical care is becoming more and more depersonalized, the patient is being treated by a series of specialists, none of whom has primary responsibility for him as a human being, and the medical profession is increasingly holding itself forth as superhuman, capable of life-saving miracles. As Professor Anne Somers recently stated: "The medical profession has been neglecting the personal aspect of health care and overlooking the role of the patient as a responsible agent in the treatment of his own illness and the maintenance of his own health." Malpractice lawyers in particular agree that the physician that develops good rapport with his patients, talks to them and tells them exactly what to expect from a course of treatment, and elicits patient participation in decision making is much less likely to be sued than the one who does not. Informed consent can become the focus for this communication and can provide the patient with the opportunity to participate in decisions concerning his health care. And this, we submit, is the genesis and continuing rationale for the doctrine. Thus, if changes in the law of informed consent reduce rather than increase physician/patient communication the result is likely to be an increase, not a decrease, in malpractice suits.

A final note in conclusion. Just as we in this article have suggested that the doctrine of informed consent was born of a need to improve the communication between physician and patient, there is also a clear need for physicians and lawyers to communicate better as well. Much of the confusion in the consent area stems from a refusal—sometimes stubborn, sometimes indignant, and often puerile—of the physician and the lawyer to try to understand the other's concerns about issues in which both have a vital interest. Informed consent involves troublesome and perplexing legal, medical, and moral questions, and there is no doubt that the problems which doctors face in discharging their obligations in this area have become increasingly more difficult. Yet, when the need for mutual trust and understanding between the professions seems greatest, attitudes have become fixed and intransigent. It is our hope that this article, written by lawyers in a medical journal, contributes to the dialogue and not the debate.

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Hypothesis: A Molecular Basis for the Hyperammonemia and Potassium Wastage Associated with Hepatic Failure

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Abstract

Plasma lysine and ornithine were found to be significantly elevated in patients with a blood ammonia of more than 200 micrograms per deciliter. Based upon this, evidence is assembled and presented implicating an increase in lysine secondary to a decrease in its degradation by the liver as the primary cause for the decreased urea production and increased ammonia formation via the mechanism of allosteric enzyme inhibition, during hepatic failure. The potassium loss, also associated with liver failure, is explained on the basis of an intracellular surrogate ion effect for potassium that lysine appears to have. This mechanism may also be applicable in the case of ornithine which bears a structural resemblance to lysine.

Introduction

Recently, patients with hepatic failure were found to have relatively elevated plasma lysine and ornithine when compared to a control group without hepatic failure. With clinical improvement, these amino acids decreased (1, 2). Hepatic coma, experimentally induced in dogs, causes the concentration of lysine and certain other amino acids to greatly increase in the cerebral cortex and blood (3).

The diabasic amino acid, lysine, when administered to human beings or animals under certain conditions, can mimic the biochemical abnormalities which are seen in hepatic failure (4). Because of the similarity between the pharmacologic effects of lysine and the abnormalities seen in hepatic failure, a study was undertaken to determine if there was an association between blood ammonia and the diabasic amino acids during hepatic failure. Evidence is also presented and reviewed implicating lysine as an etiologic agent causing some of the abnormalities seen in hepatic failure.

Materials and Methods

Ammonia determinations were performed according to the method of Seligson and Hirahara as modified by Reinhold and Chung (5).

Plasma lysine and ornithine were determined on the Durrum-500 amino

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acid analyzer via a modification of the method developed by Moore and Stein (6).

Patients were selected for the study by reviewing the daily blood ammonia determinations performed at Beth Israel Hospital between January and August of 1974. Determinations were generally performed on chilled, heparinized venous blood; in a few instances, arterial blood was used, as noted in Table I. All patients with a blood ammonia exceeding 200 μg/dl were included. All were in hepatic coma at the time the blood was taken. Concomitantly, a number of patients in hepatic coma and precoma with blood ammonia values of less than 200 μg/dl were studied. Also included were the two original cases from 1972, and one patient with delirium tremens and an elevated arterial ammonia. Blood ammonia levels were then obtained on a group of patients who were comatose and free of liver disease. Plasma lysine was determined on all of the patients, frequently on the same blood sample that was used for analysis of the blood ammonia, or on a sample drawn within six to eight hours of the blood ammonia. Plasma ornithine was determined on most of these patients at the same time. If serum K^+ or BUN were done within 24 hours of the blood ammonia the results were included in Table I.

All of the patients studied were receiving no food by mouth and were maintained on intravenous feeding. None received purified amino acid supplementation. On occasion, albumin had been given to some.

Results

Table I consists of a tabulation of the clinical data as indicated. On inspection it can be seen that the highest plasma lysine values occur in those patients with an elevated venous ammonia. Of those with a venous blood ammonia above 200 micrograms per deciliter, none had a plasma lysine below 24 micromoles per deciliter. In the three patients who had *arterial* blood ammonia determinations above 200 micrograms per deciliter, the plasma lysine was 21 micromoles per deciliter or less. In five out of seven of the samples with elevated blood ammonia, plasma ornithine was also elevated. No conclusions could be drawn regarding the effect on serum potassium or the blood urea nitrogen.

In the control group of patients with coma for a variety of causes but free of liver disease, the plasma lysine was 18 micromoles per deciliter or less and the plasma ornithine was less than 13 micromoles per deciliter in 4 patients, and 14 micromoles in one patient (the upper limits of normal in adults for plasma ornithine is 13 micromoles per deciliter).

Interpretation of Results

Plasma Lysine: On a regular diet the upper limit of normal for plasma lysine is 24 micromoles per deciliter. During fasting or while on a low protein diet, 18 micromoles per deciliter is the upper limit of normal (7, 8). In animal studies, intracellular lysine exceeds extracellular lysine tenfold. In the presence of potassium depletion, intracellular lysine is forty times greater than extracellu-

TABLE I

Patient	Age & Sex	Mental Status	Serum K^+ mEq/l	BUN mg/dl	Blood Ammonia µgm/dl	Plasma Lysine µmoles/dl	Plasma Ornithine µmoles/dl
			Patient in Hepatic Coma				
1.	35 M	Coma	4.6	25	500	32	28
2.	60 M	Coma	4.3	59	1,647	54	17
3.†	69 F	Coma	5.2	16	210	25	11
4.	44 M	Coma	3.9*	12	234	32	18
4.	—	Coma	4.5*	Not done	235	29	12
4.	—	Coma	5.1*	81	216	110	27
4.	—	Coma	4.9*	84	67	77	16
5.	32 F	Coma	3.1*, ‡	.5	132	30	10
6.†	64 M	Coma	5.2*	14	120	57	16
7.	30 F	Coma	3.6	11	235**	21	12
7.	—	Coma	Not done	Not done	208	24	11
8.	70 F	Coma	Not done	Not done	100	21	20
8.	—	Coma	4.2	45	265**	16	12
8.	—	Coma	3.3	51	221**	18	15
9.	34 M	Coma	Not done	Not done	81	19	8
10.	63 F	Precoma	3.8	32	61	17¶	16¶
11.	25 F	Precoma	3.3	10	143	12	14
12.	M	Coma	Not available	Not available	79	14	12
13.	40 M	Precoma	Not available	Not available	191	18	11
14.	43 M	Del. Trem.	3.9	6	210**	12	2
			Patients in Coma without Liver Disease				
1A	81 M	Coma	5.0	20	Not done	7	
2A	56 F	Coma	3.1	46	Not done	15	8
3A	77 F	Coma	4.2	19	102	11	7
4A	F	Coma	5.0	36	109	18	11
5A	44F	Coma	5.7	61	45	11	14
6A	70 M	Coma	4.6	19	Not done	14	9

* Potassium supplementation just prior to or on the day that serum K^+ was determined.

† Original index patient.

‡ Patient receiving spironolactone.

** Arterial blood ammonia.

¶ Amino acid determinations performed on serum.

lar lysine (4, 9, 10). Consequently, if hypokalemia and potassium depletion are present, as is frequently the case during hepatic failure, plasma lysine may not reflect the intracellular concentration, and, in reality, the level within the cell is increased.

A falsely elevated plasma lysine could possibly occur if hemolysis were present. Lysine and ornithine determinations were only done on nonhemolyzed blood samples.

Blood ammonia: The normal blood ammonia levels utilizing the method mentioned in this study range from 50 to 150 micrograms per deciliter. With serial determinations on a given sample, the precision factor is 10%.

In hepatic disease, arterial ammonia can exceed venous ammonia by as much as 60%. In one study, 15 out of 26 patients had arterial ammonia levels exceeding venous ammonia by 40% or more. Only 4 patients had a difference of less than 10% (11).

Ammonia is most soluble at low temperatures; if the blood is not kept in a chilled state during transportation to the laboratory, falsely low values may be found. On the other hand, an increase in atmospheric ammonia in the lab can deceptively cause an elevation in blood ammonia.

Discussion

Urea and ammonia synthesis: A diminished blood urea nitrogen frequently accompanies hepatic disease, even while the liver is relatively well compensated (12–15). In cirrhosis without hepatic failure an increase in protein loading results in a subnormal rise in urea production (16). In animal studies to achieve a comparable drop in urea, 85% or more of the liver must be removed (17, 18). This indicates that the decreased blood urea nitrogen seen in hepatic disease is not analogous to the experimentally induced state where the liver is extirpated.

It has been known for many years that the addition of lysine to the diet of cattle results in a drop in urea excretion (19). Administration of lysine to uremic dogs results in a drop in the blood urea (20, 21), and lysine given to azotemic patients has been found to have the same effect (22). Recently, clinicians have rediscovered this phenomenon. A combination of essential amino acids containing lysine is presently being administered to patients with azotemia and has been reported to result in a drop in BUN and urea excretion (23, 24).

The association of hyperammonemia and hepatic failure is undisputed. Patients with cirrhosis have been found to have an increase in the excretion of the ammonium ion via the urine while in a relatively well-compensated state (15). To date the search for the cause of this phenomenon has not been very fruitful. Glutamine, the amino acid which is normally thought to be a precursor for ammonia, is not elevated in hepatic failure associated with hyperammonemia (25), and may even be decreased (1).

The breakdown of nitrogenous substances by gut bacteria has been well documented and has been evoked as a theory to explain the occurrence of increased blood ammonia values in hepatic failure with coma (26), but substantiation has not been adequate. Hydrogen ion concentration is an important variable in the absorption of ammonia from the gut. An acid pH within the colonic lumen favors the retention of ammonium ions within the colonic contents. Only when there is an overwhelming gradient induced by the administration of ammonium ions is there significant absorption of ammonia (27).

When lysine is fed to cirrhotic patients there is an increase in blood ammonia. Other amino acids may have the same effect. (28) In rats, lysine causes an increase in ammonia formation (46). Congenital lysine intolerance, which is caused by a defect in lysine degradation, is associated with the development of hyperammonemia following the ingestion of lysine-rich foods or free lysine (29, 43). Recently Reyes Syndrome, which has hyperammonemia as one of its components, has been found to be associated with an elevated blood lysine (31, 32). Patients with hepatic failure who were undergoing charcoal dialysis were shown to have an elevated blood lysine and blood ammonia. After dialysis there was a significant drop in the blood lysine as well as in other amino acids. During dialysis there was a gradient across the dialysis column, indicating that absorption of the lysine upon the column was taking place; this held true for other amino acids also. Ammonia was not absorbed, but a drop in blood ammonia occurred a few hours later (2).

Potassium metabolism: Hepatic failure may be associated with an increase in potassium excretion with or without concomitant hypokalemia in the absence of diuretic therapy or hyperaldosteronism (4). The rate of loss can be so great that active secretion of potassium by the kidneys takes place. Usually, potassium secretion is seen only during potassium loading or in the presence of azotemia (33).

Lysine administration to animals has been shown to cause potassium secretion by the kidneys. When given intravenously to dogs, potassium clearance by the kidneys exceeds creatinine clearance, indicating active potassium secretion by the kidneys. This occurs in the absence of potassium feeding. Ornithine has a similar effect (34, 35). Lysine feeding also has the same effect in rats (9).

In man, hyperalimentation with amino acid solutions containing large amounts of lysine has also been associated with excessive urinary potassium loss, indicating active potassium secretion by the kidneys (36, 37).

The Molecular Basis for the Effects of Lysine

According to the evidence thus far presented, the existence of a lysine, urea, ammonia, potassium relationship or axis cannot be disputed. What remains unexplained is the mechanism whereby this interrelationship takes place.

In 1944, Hunter and Downs, while working with arginase, discovered that a mixture of amino acids, when added to an arginine-arginase solution, inhibited the formation of urea. Upon investigation, the two amino acids which were most active as inhibitors were lysine and ornithine (38). Subsequently, this inhibitory effect was documented by others also (29). Using semiquantitative techniques, the original authors identified the inhibition as being of a competitive type. A reanalysis of the original data supplied by Hunter and Downs, using a different analytic technique, unequivocally substantiates the contention that lysine is a competitive inhibitor of arginase (Fig. 1 and Table II).

The Allosteric Theory

From 1963 through 1965, Jacques Monod and his coworkers published a series of articles analyzing the problem of enzyme inhibition. Evidence was presented showing that inhibition takes place when an inhibitor binds to a

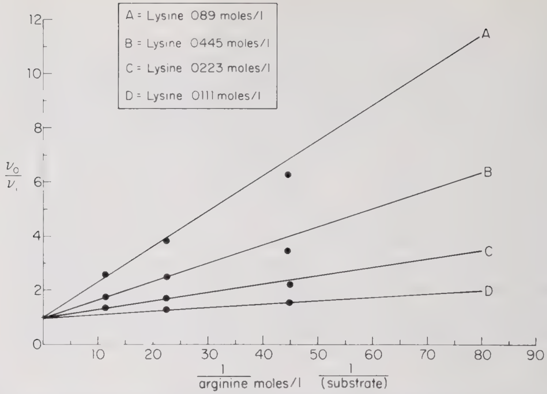

Fig. 1. This figure represents a replot of the data supplied by Hunter and Downs. $\mathcal{V}_o$ is the initial velocity of the reaction without lysine (inhibitor). $\mathcal{V}_i$ is the initial velocity in the presence of lysine. When $\frac{\mathcal{V}_o}{\mathcal{V}_i}$ vs 1/substrate is plotted for various concentrations of lysine, then a series of graphs is obtained which approaches linearity at high concentrations of substrate. These lines obviously intersect at the same point on the ordinate where the initial velocity with and without the inhibitor are equal. This point also represents the maximal velocity for the reaction since the concentration of substrate is infinite.

Proof that a plot of $\frac{\mathcal{V}_o}{\mathcal{V}_i}$ vs $\frac{1}{S}$ is a straight line for high concentration of substrate with the slope being a function of the inhibitor concentration and intersecting the Y axis at 1 in the case of competitive inhibition (K type inhibitors) is given below.

Given:

I = Inhibitor
S = Substrate

$$1)\ \mathcal{V}_0 = \frac{V_{\max}}{1 + K_m/S}$$

$$2)\ \mathcal{V}_i = \frac{K_m\,(1 + I/K_i)}{V_{\max}}\ \frac{1}{S} + \frac{1}{V_{\max}} \qquad \text{(ref 56)}$$

Then, we can combine these two equations and get:

$$\frac{\mathcal{V}_0}{\mathcal{V}_i} = \frac{V_{\max}\text{ with Inhibitor}}{V_{\max}\text{ without Inhibitor}} + \frac{K_m}{K_i} \cdot \frac{I}{K_m + S}$$

In the case of competitive inhibitors, we know that $V_{\max}$ with Inhibitor equals $V_{\max}$ without Inhibitor; therefore,

$$\frac{V_{\max}\text{ with Inhibitor}}{V_{\max}\text{ without Inhibitor}} = 1$$

For competitive inhibitors, the above formula now simplifies to:

$$\frac{\mathcal{V}_0}{\mathcal{V}_i} = 1 + \frac{K_m}{K_i} \cdot \frac{I}{K_m + S} \qquad \text{(ref 56)}$$

If the concentration of the inhibitor is held constant and only the substrate is varied, then for high

TABLE II

Tabulation of the Data by Hunter and Downs (ref. 38), Used by the Author to Prepare the Graphs in the Manuscript.

Lysine moles/l (Inhibitor)	Arginine moles/l (Substrate)	$\frac{1}{\text{Arginine moles/l}}$	$\frac{v_1}{v_0}$	$\frac{v_0}{v_1}$
.0890	.0223	44.84	.160	6.25
.890	.0445	22.47	.260	3.85
.0890	.0890	11.24	.393	2.54
.0445	.0223	44.84	.290	3.45
.0445	.0445	22.47	.40	2.50
.0445	.0890	11.24	.57	1.75
.0223	.0223	44.84	.454	2.20
.0223	.0445	22.47	.590	1.69
.0223	.0890	11.24	.734	1.36
.0111	.0223	44.48	.638	1.57
.0111	.0445	22.47	.740	1.35

specific inhibitor-receptor site that is engineered into the protein structure of the enzyme. Attachment of the inhibitor at this site induces a conformational change in the enzymatic protein which results in a quantitative change in the enzyme's capabilities, causing a change in the $V_{\max}$ or the K_m. These inhibitors are respectively termed V type and K type inhibitors (41), with competitive inhibitors frequently being of the K type. The inhibitor substances have no structural similarity to the enzyme substrate and are appropriately called allosteric inhibitors. Frequently, these allosteric inhibitors are end-products of a chain of biochemical reactions which at some point is mediated by the enzyme undergoing inhibition, but this need not be so (39). Analogs of the allosteric inhibitor may also have the ability to react with the allosteric receptor site (40). Enzymes which have the potential for allosteric inhibition are thought to exist in two states of quaternary configuration, the R state and the T state. Both of these states are in equilibrium and can be expressed by a specific equilibrium constant. (This constant is not to be confused with the substrate-enzyme equilibrium constant.) If equilibrium favors the T state, or if allosteric inhibitor binding occurs which causes displacement of the enzyme to the T state, the enzyme manifests sigmoid behavior when velocity is plotted against substrate. Because of the cooperative effect, the initial substrate unit facilitates the binding of a subsequent substrate unit on the enzyme. If equilibrium favors the R state and in the absence of the allosteric inhibitor, the enzyme exhibits the classic Michaelian hyperbolic function in the velocity vs substrate plot (41, 42).

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concentrations of substrate, the equation can be simplified to:

$$\frac{K_m}{K_i} \cdot \frac{1}{S} + 1 = \frac{\mathcal{V}_0}{\mathcal{V}_i}$$

Now a plot of $\frac{\mathcal{V}_0}{\mathcal{V}_i}$ vs $\frac{1}{S}$ will approach a straight line. The line will intersect the ordinate (Y axis) at 1 in the case of competitive inhibition with the slope dependent on $\frac{K_m}{K_i}$.

Lysine and Ornithine as Allosteric Inhibitors

Lysine bears a structural similarity to ornithine but is quite dissimilar when compared to arginine (Fig. 2). Allosteric inhibition of arginase by ornithine would appear to be logical since ornithine is the end-product when arginine is acted upon by arginase to form urea (Fig. 3). Lysine, an analog of ornithine, would be expected to have a similar effect. Analysis of the original data supplied by Hunter and Downs is consistent with a change to sigmoid kinetics when lysine is added in increasing amounts to an arginine and arginase mixture (Fig. 4). It would have been more accurate to have plotted velocity vs substrate but the ratio $\frac{v_i}{v_o}$ is just as valid to identify the shape of the curve (see proof accompanying Figure 4). Clinical evidence for intrahepatic arginase inhibition also exists. One group of investigators found that in hepatic coma with hyperammonemia, there is a decrease in intrahepatic arginase "activity" (30). To date, the intrahepatic urea cycle enzyme analysis for the other enzymes during hepatic failure has not been done. Even if they were to be performed, interpretation would be difficult since experimental evidence

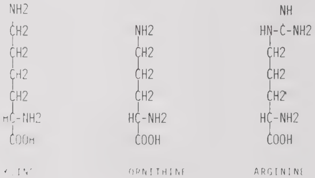


Fig. 2

UREA SYNTHESIS IN MAN

$$\text{AMMONIA} + \text{CARBON DIOXIDE} + \text{ATP} \xrightarrow{\textit{carbamyl phosphate synthetase}} \text{CARBAMYLPHOSPHATE} + \text{ADP}$$

$$\text{CARBAMYL PHOSPHATE} + \text{ORNITHINE} \xrightarrow{\textit{ornithine carbamyl transferase}} \text{CITRULLINE} + \text{PHOSPHATE}$$

$$\text{CITRULLINE} + \text{ASPARTIC ACID} + \text{ATP} \xrightarrow{\textit{arginosuccinate synthetase}} \text{ARGINOSUCCINIC ACID} + \text{ADP}$$

$$\text{ARGINOSUCCINIC ACID} \xrightarrow{\textit{arginosuccinate cleavage enzyme}} \text{ARGININE} + \text{FUMARIC ACID}$$

$$\text{ARGININE} \xrightarrow{\textit{arginase}} \text{UREA} + \text{ORNITHINE}$$

Fig. 3.

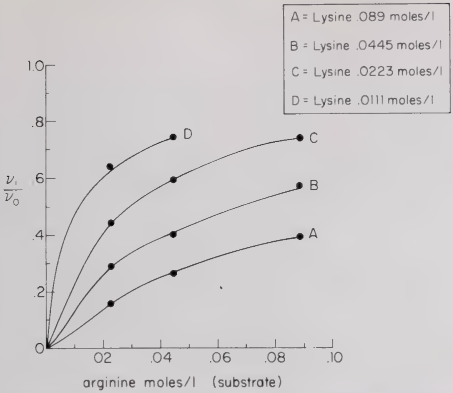


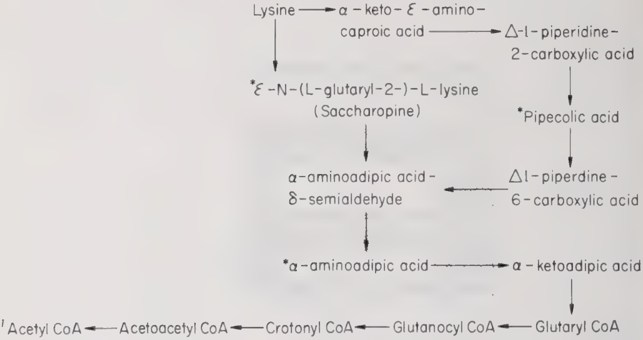
Fig. 4. Proof that the configuration of the graph $\mathcal{V}_i/\mathcal{V}_o$ vs S is similar to the curve generated by the plot $\mathcal{V}_i$ vs S in the case of sigmoid-shaped curves.
1) The enzyme arginase follows classic Michaelis and Menten kinetics, a plot of the initial velocity vs substrate, yields a hyperbolic curve approaching the limit in an asymptotic fashion for initial velocity greater than zero, the limit being $V_{\max}$.
2) The reciprocal of a hyperbolic function is a linear function. Therefore, if a sigmoid function is divided by a hyperbolic function, the sigmoidality of the curve will be maintained and the only change that would take place would be that there would be a change in slope.
Then:
The curve generated by $\mathcal{V}_i/\mathcal{V}_o$ vs S will differ from the curve $\mathcal{V}_i$ vs S by a change of slope.

exists both in primates and rats that the enzymes mediating urea formation are inducible and substrate-dependent (16, 30, 44, 45). Therefore, a decrease in their concentration could occur with an enzymatic block at some point in the cycle (46). Phylogenetic evidence also exists, indicating that lysine plays a role in regulating urea formation. The amphibian *Rana cancrivora*, when acclimated to salt water, produces urea to regulate intracorporeal osmotic pressure. Acclimation to fresh water is associated with a reduction both in muscle and blood urea. Concomitantly, there is a dramatic increase in blood lysine while all the other amino acids are decreased and the enzymes needed for the urea cycle are still present (51).

Lysine Degradation and the Theoretical Basis for the Effects of Lysine on Potassium Balance

In man lysine is degraded to alpha aminoadipic acid semialdehyde, which is broken down further to form acetyl CoA (Fig. 5). An intermediary before the formation of the semialdehyde is saccharopine (29, 43). The enzyme needed to convert lysine into saccharopine is found only in the liver (48–50). A normally functioning human liver has been estimated to be able to degrade 114 g of lysine each day (47). It is not inconceivable that hepatic failure is associated with the loss of the ability by the liver to degrade lysine. Concomitantly,

PROBABLE PATHWAYS OF
LYSINE DEGRADATION IN MAN


Lysine
α-keto-ε-amino-caproic acid
Δ-1-piperidine-2-carboxylic acid
*ε-N-(L-glutaryl-2-)-L-lysine (Saccharopine)
*Pipecolic acid
α-aminoadipic acid-δ-semialdehyde
Δ1-piperdine-6-carboxylic acid
*α-aminoadipic acid
α-ketoadipic acid
1 Acetyl CoA
Acetoacetyl CoA
Crotonyl CoA
Glutanocyl CoA
Glutaryl CoA

*These compounds have been found in man

[1] This last step is presumed to be correct since patients with hyperlysinemia when given radioactive lysine in balance studies have a decreased production of labeled Co_2, compared to control patients Ref. 43

Known to occur in liver tissue of man Ref. 47

Fig. 5.

feedback amplification of the inability to degrade lysine could be caused by the presence of the ammonium ion which has been shown to completely inhibit the breakdown of lysine into saccharopine (52). As mentioned earlier, intracellular lysine can be from 10 to 40 times greater than extracellular lysine. An increase in intracellular lysine may result either from potassium depletion (9) or lysine excess (34, 35). In either case, the final result is the displacement of potassium within the cell by lysine. Lysine has a pKa of 9 for the amino group in the epsilon position. Intracellularly, the hydrogen ion concentration is much higher than the extracellular concentration. Experimentally, the pH has been found to be between 6 and 7.0 (10, 53, 54). Virtually all of the lysine is positively charged at the intracellular pH and undoubtedly behaves in the fashion described by the Gibbs-Donnan theory, resulting in the displacement of potassium from within the cell and a resultant kaluresis (4, 35). Undoubtedly an additional factor which acts to potentiate the potassium loss is the passage of ammonia into the cell. The cell membrane is impermeable to the ammonium ion; only free ammonia can pass through the membrane (26, 55). Ammonium hydroxide has a pKB of 9.4. Utilizing the Henderson-Hasselbach equation it can be calculated that at a pH of 7.7 the ratio of NH_3 to NH_4^+ is 1:50 at 7.4 the ratio is 1:100 and at a pH of 7.0 the ratio is 1:250. Therefore, during hepatic failure which is frequently associated with extracellular alkalosis and a relative intracellular acidosis, the presence of a transcellular hydrogen ion gradient leads to the sequestering of ammonium ions within the cell, thereby further potentiating potassium loss. Urinary excretion of ammonium ions also depends upon the existence of a hydrogen ion gradient between the blood and renal tubular fluid (26). Blood ammonia concentration reflects the interaction of these variables in association with the rate of ammonia production.

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Diverticular Disease of the Terminal Ileum

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Diverticular disease of the distal ileum is seldom diagnosed radiologically. However, the more frequent use of pressure spot films of the right lower quadrant as a routine in all small bowel studies has increased the number of such cases described in the literature in the recent past (1).

Material

Four new cases recently diagnosed as having diverticular disease of the terminal ileum are the subject of the present paper. One of these patients had a superimposed inflammation of the diverticula with abscess formation. The first three patients in the series were asymptomatic as far as the right lower quadrant was concerned, and the diagnosis was noticed incidentally during a small bowel series. Two of these patients were noted to have coexistent diverticulosis of the ascending colon. None of the four cases demonstrated diverticula in other portions of the small bowel.

Case No. 4 was a 51-year-old woman who was admitted to the hospital with a four-day history of intermittent right lower quadrant pain. No fever or vomiting were present. Physical examination showed tenderness in the right lower quadrant, with an ill-defined mass. The symptoms subsided, but there was residual tenderness in the right lower abdomen. Radiographic examination of the small bowel showed numerous small- and medium-sized outpouchings along the medial wall of the terminal ileum (Fig. 4 A). There was evidence of a right lower quadrant mass causing a slight separation of the bowel loops in this region. Furthermore, there was a constant shallow concavity of the medial wall of the cecum (Fig. 4 A & B). At surgery, there was an inflammatory mass in the mesentery of the ileum and ascending colon. An ileocolic resection was performed. The specimen showed evidence of a perforated ileal diverticulum with subacute inflammatory changes in the mesentery.

Discussion

Although diverticular disease is often asymptomatic, as in our first three cases, inflammation, volvulus, and obstruction can be superimposed and can cause serious complications (2). To date, only nine previous cases of terminal ileal diverticulitis have been described in the radiologic literature (1, 3, 4).

Primary diverticula of the small bowel are thought to be due to herniation of the mucosa through a weakness in the bowel wall at a point of vascular

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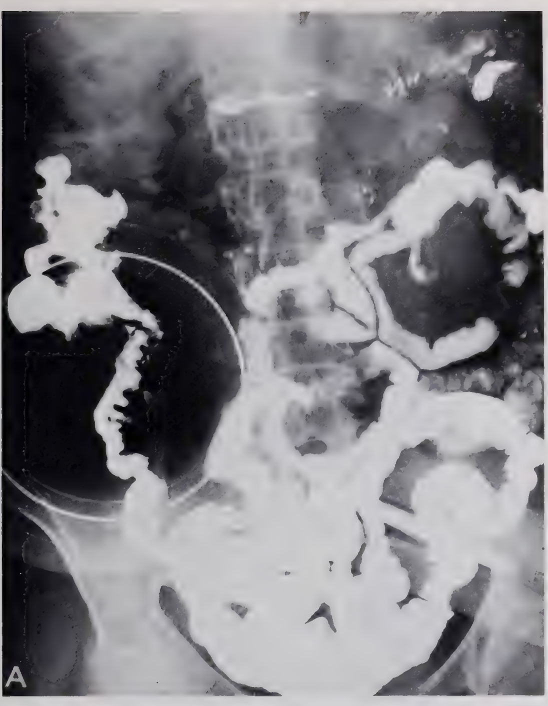

FIG. 1 A. Prone film of the small intestine with a pressure device in the right lower quadrant reveals numerous small- and medium-sized diverticula along the medial aspect of the distal 10 centimeters of ileum.

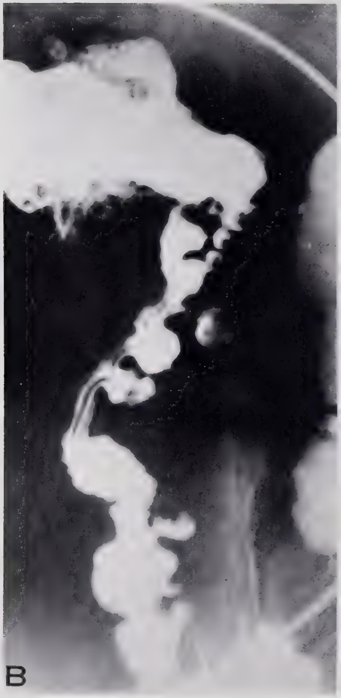

FIG. 1 B. Pressure spot film of the terminal ileum shows the narrow-necked multiple diverticula on the mesenteric surface of the terminal ileum.

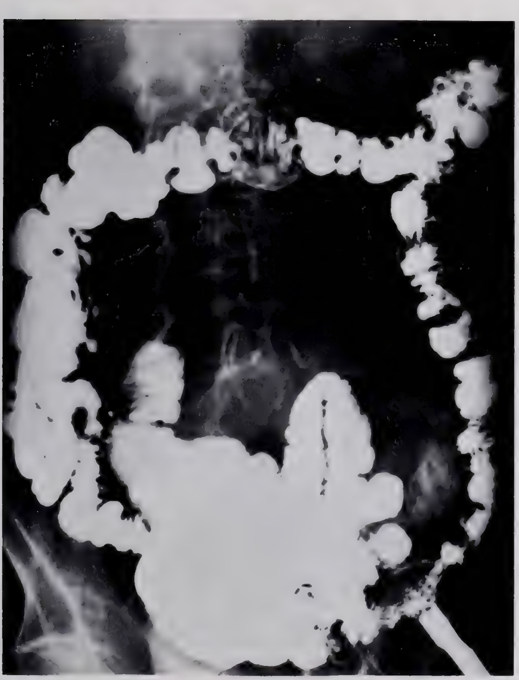

FIG. 2. Right anterior oblique view of the barium-filled distal small bowel and colon demonstrates multiple small diverticula along the medial border of the terminal 8 centimeters of ileum. Also noted are medium-sized diverticula in the ascending colon.

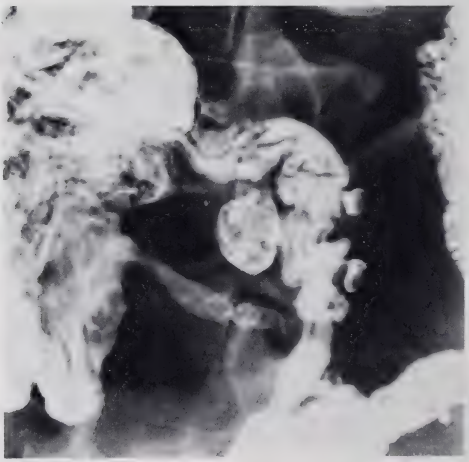

FIG. 3. Pressure spot film of the ileocecal region shows multiple small diverticula on the medial border and one larger diverticulum on the lateral side of the terminal ileal loop.

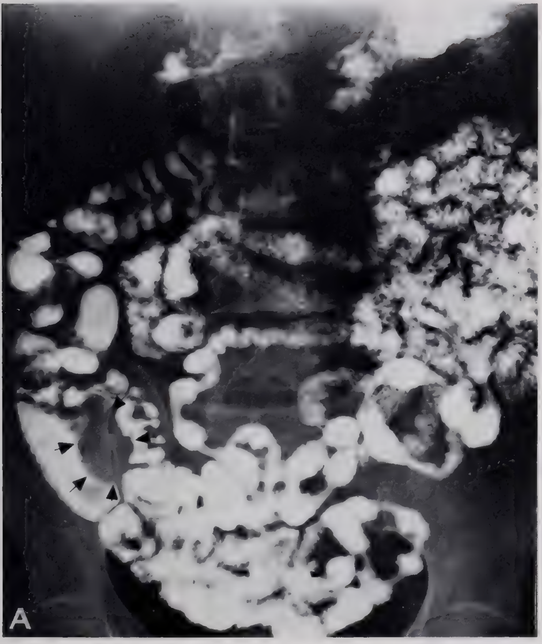

FIG. 4 A. Supine film of the abdomen in the late stages of a small bowel series shows a few diverticula medially along the terminal 5 centimeters of the ileum. There is evidence of a small mass separating the terminal ileal loops from the cecum (along arrows). The medial wall of the cecum is concave and slightly pleated, but no ulceration or nodularity is demonstrated.

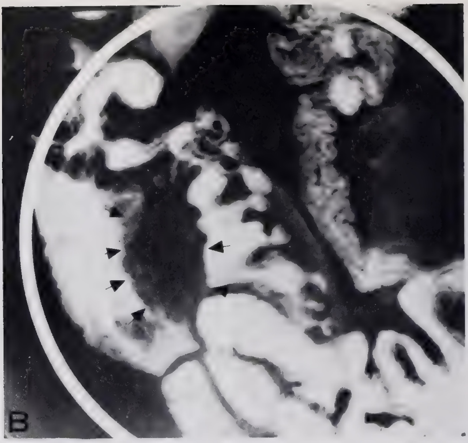

FIG. 4 B. Pressure spot film of the same region demonstrates the necks of the diverticula along the medial wall of the terminal ileum (along arrows). There appears to be a poor filling of small diverticular necks on the inferior border of the terminal-most ileum, along the superior edge of the ileocecal mass. This may represent the inflamed diverticula and the probable source of the surrounding abscess.

entry. In the terminal ileum, these diverticula are usually small, multiple, and have narrow necks (Figs. 1, 2, and 3). Meckel's diverticula, on the other hand, are larger, solitary, antimesenteric, and usually occur 30 to 90 centimeters proximal to the ileocecal valve. Most of the primary terminal ileal diverticula are situated on the mesenteric side, but in about 10% of the cases they can be identified on two opposite rows along the ileal lumen (1).

Summary

Three cases of terminal ileal diverticulosis and one of diverticulitis with abscess are presented and discussed.

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Tetracycline Toxicity Presenting as a Multisystem Disease

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Abstract

A 24-year-old woman patient with an 8-year history of tetracycline intake had clinical and chemical findings suggestive of a multisystem disease. The organ systems involved included liver, kidney, brain, hematopoietic system, and possibly the pancreas. After discontinuing tetracycline, the symptoms abated and the hepatorenal function tests, abnormal EEG, and hemolytic anemia showed normalization or marked improvement. The diabetes mellitus noted in this patient was probably a coincidental finding although the patient had no family history of diabetes.

This case emphasizes the improtance of careful evaluation of patients before this drug is given as well as the dangers of its indiscriminate use.

The pertinent literature of tetracycline toxicity is reviewed in relation to the toxic effects in this patient.

The tetracyclines have enjoyed widespread use in various clinical situations since their recognition as a pharmaceutical agent in 1948. During this period of time, many adverse effects of this drug have been reported (1–29). The following case presentation includes clinical and chemical findings of multisystem involvement, most of which were due to tetracycline toxicity.

Case Report

A 24-year-old white woman was admitted to Beth Israel Medical Center with multiple complaints of eleven months duration, including dizziness, pains in her extremities with leg swelling, anorexia, nausea, and frequent bowel movements. The patient has a history of severe facial acne diagnosed at age 16 and was taking oral tetracyclines prophylactically 1 gm per day, with occasional intakes of 3 gms per day, for eight years until the day of present admission.

In 1959, she sustained a cerebral concussion and an abnormal electroencephalogram (EEG) was noted which supposedly reverted to normal several years later. In 1972, the patient had an episode of hives and was told she had angioneurotic edema. Tests done at that time revealed multiple allergies, and she was treated symptomatically with antihistamines. Three months prior to admission, she was told she had diabetes mellitus and was placed on phenformin which was discontinued after several days due to the development of nausea and vomiting. There is no family history of diabetes mellitus and the patient denied any photosensitivity.

Physical examination revealed a well-developed, thin white woman in no acute distress. The supine blood pressure was 105/65, with a pulse of 90/min without orthostatic changes. Skin of the face was clear without evidence of acne but was indurated and revealed small pitted scars. The rest of the physical examination was entirely within normal limits except for conjunctival pallor.

Initial laboratory studies included a hemoglobin level of 8.8 g%, with a hematocrit of 26.8%, a white blood cell count of 8500 with a normal differential, a blood urea nitrogen (BUN) of 51 g%,

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serum sodium of 134 mEq/L, serum potassium of 4.5 mEq/L, serum chloride of 100 mEq/L, serum glucose of 97 mg%, and a serum bicarbonate of 26 mEq/L. Urine analysis was normal. There was no proteinuria, and the urinary sediment was free of red blood cells, white blood cells, and casts. The urine specific gravity was 1.015.

By the sixth hospital day her anemia worsened, necessitating transfusion of two units of packed red blood cells. Indices were normocytic and normochromic. Serum iron was 135 μg/100 ml with a total iron binding capacity of 270 μg/100 ml. Serum B_{12} was 1100 pg/ml, serum folate was 9 ng/ml, serum haptoglobin was <25 mg/100 ml, serum lactic dehydrogenase was 220 IU/L, serum creatinine was 3.3 mg/100 cc, creatinine clearance was 25 cc/min, total protein was 5.1 gm/100 cc, serum albumin was 2.8 g/100 cc, total bilirubin was 0.7 mg/100 cc, serum glutamic oxaloacetic transaminase 70 Karmen U/ml, and serum glutamic pyruvic transaminase was 86 Karmen U/ml. The first Coombs test was positive but was followed by several negative tests. Tests for occult blood in the stool and for urine hemosiderin were repeatedly negative. Bone marrow was normal with adequate iron stores. An abnormal EEG was recorded on the second hospital day showing nonfocal, bilateral cerebral dysfunction. An electromyogram done on the seventh hospital day indicated myopathic involvement of the lower extremities. However, a muscle biopsy was normal. A 24-hour urine collection showed normal urinary electrolytes with 5 mg/24 hours of protein and no aminoaciduria. Lumbar puncture and brain scan were normal. X-rays of the chest, skull, right leg, upper gastrointestinal series, barium enema and intravenous pyelogram were normal. Rast scores and serum IgE levels were normal, and skin tests were positive for mumps and negative for first strength PPD and coccidiomycosis. A serum protein electrophoresis revealed an albumin of 5.05 g/100 ml, α 1 0.41 g/100 ml, α 2 1.12 g/100 ml, β 0.54 g/100 ml and γ globulin of 0.88 g/100 ml. On the tenth hospital day, an abnormal glucose tolerance test was found with a fasting blood sugar of 80 mg%, a one-hour serum glucose of 208 mg%, a two-hour serum glucose of 196 mg%, a three-hour serum glucose of 168 mg%, and a five-hour serum glucose of 102 mg%. Corresponding insulin levels were: 39 μU/ml fasting, 43 μU/ml at one hour, 64 μU/ml at 2 hours, 64 μU/ml at three hours, and 23 μU/ml at five hours. Antinuclear antibody (ANA), LE tests, serum complement, and cryoglobulins were all repeatedly negative or within normal limits.

Oral tetracyclines were disontinued on the twentieth hospital day and erythromycin, 250 mg PO QID was substituted. Serum tetracycline levels were drawn at this time and a value of 22.5 mg/L was reported, an extremely high level (therapeutic level 1.5 – 10 mg/L). Following the discontinuation of tetracycline, marked improvement was noted subjectively. The hepatic and renal function tests gradually fell to normal levels. However the patient still complained of vague leg pains associated with mild swelling. Diabetic neuropathy was considered as a possible cause and she was started on Dilantin® which was subsequently discontinued due to the development of a diffuse papular eruption. The patient also remained anemic, and was again transfused with two units of packed red blood cells. Posttransfusion, the hemoglobin was 10.1 g%, and remained at this level at the time of discharge. At discharge the reticulocyte count was 4%, with a serum haptoglobin of 50–100 mg%. A repeat glucose tolerance test showed a fasting glucose of 107 mg%, a one-hour value of 271 mg%, a two-hour value of 322 mg%, and a three-hour value of 206 mg%.

The electroencephalogram was repeated on the 36th hosptial day and was still abnormal but with significant improvement compared with earlier tracings. Repeat tetracycline levels drawn two weeks after discontinuation of tetracycline were much lower, 3.99 mg/L, and one week prior to discharge a very low 0.17 mg/L. After discharge from the hospital, the patient's red cells were challenged with tetracycline by the method of Lalezari (22). The patient had a strong positive reaction in a 1:40 dilution to tetracycline. Rechallenge of patient's red blood cells three months after discontinuing tetracycline revealed no antibodies against this drug.

Pancreatic secretion studies done after discharge revealed a normally functioning exocrine pancreas. The blood amylase was normal and there was no evidence of pancreatic duct obstruction.

Discussion

Toxic effects of tetracycline administration have been reported. Many of these represent relatively acute toxicity usually limited to a single organ. Our

TABLE I

Results of Hematology Studies

Date	Serum Haptoglobin mg%	Hemoglobin g/100 cc	RBC/cu mm	Hematocrit Level %	WBC/cu mm	Reticulocytes %	Sedimentation Rate Westergren	Bone Marrow
9/10/74	<25	8.8	2.47	26.8	8500	—	10	—
9/16/74	—	7.1	—	23	7400	2.1	10	Normal
9/16/74	Transfused Two Units Packed Cells							
9/17/74	—	11.0	—	31.5	7800	—	—	—
9/25/74	<25	—	—	—	—	—	—	—
9/30/74	Oral Tetracycline Discontinued							
10/7/74	—	7.8	—	24.1	7700	0.7	—	—
10/8/74	50-100	Transfused Two Units Packed Cells						
10/15/74	—	10.6	—	32.2	7900	—	—	—
10/18/74	—	10.4	—	30.9	7400	3	28	—
10/21/74	—	9.7	—	29.0	6100	8	38	—
10/23/74	—	10.2	—	30.8	7500	4	—	—

TABLE II

Results of Blood Chemistry Studies

Date	BUN mg/100 cc	Creatinine mg/100 cc	Creatinine Clearance cc/min	Total Protein gm/100 cc	Albumin gm/100 cc	Glucose mg%	Total Bilirubin mg/100 cc	Alkaline phosphatase I Units/l	Lactic Dehydrogenase I Units/l	SGOT Karmen Units/ml	SGPT Karmen Units/ml	Glucose Tolerance Test	Tetracycline Levels mg/l
9/10/74	51	2.8	25	5.7	2.8	100	0.7	90	220	60	—	—	—
9/16/74	62	3.3	—	4.8	2.7	120	0.5	75	130	70	86	—	—
9/20/74	—	—	—	—	—	—	—	—	—	—	—	Abnormal	—
9/30/74	Oral Tetracycline Discontinued												
10/3/74	40	2.3	—	5.4	3.0	90	0.6	70	190	75	—	—	22.5
10/15/74	20	1.5	50	6.4	4.0	110	0.5	50	170	25	32	Abnormal	0.17

patient had an eight-year history of tetracycline ingestion with toxic involvement of the kidney, liver, brain, blood, and possibly the pancreas. It seems appropriate at this time to review the pertinent literature dealing with these effects.

Several reports have documented the nephrotoxic effects of degraded tetracycline in the production of a reversible Fanconi syndrome (1,2). The biochemical abnormality of the adult Fanconi syndrome is one of a generalized tubular defect and includes proteinuria, renal glycosuria, generalized aminoaciduria, high phosphate and uric acid clearances with reduced plasma levels of these two substances, reduced urinary concentrating and acidifying ability and abnormal urinary potassium losses (3). It was demonstrated in rats that anhydro-4-epitetracycline was the only degradation product causing urinary findings similar to the Fanconi syndrome (4). The other two degradation products, namely anhydrotetracycline and epitetracycline produced no urinary findings suggestive of a tubular defect. When anhydro-4-epitetracycline was given at a lower dosage (275 mg/Kg) in combination with the other degradation products no harmful effects were seen in the rat, indicating that the degree of impairment was most likely a function of the dose and duration of administration of anhydro-4-epitetracycline. In vitro, tetracyclines have been shown to inhibit oxidative phosphorylation and mitochondral respiration in the renal cells causing mitochondria to swell (1). They seem to act as a chelating agent as its effects are reversible with magnesium salts. The possiblity that degraded tetracycline directly blocks tubular reabsorption sites thereby causing a Fanconi-like syndrome cannot be excluded.

Since Bateman's first report of azotemia associated with oxytetracycline (5), others have described similar findings (6,7). Shils attributed the azotemia to an anti-anabolic effect (8). He pointed out that when tetracyclines are administered in usual therapeutic doses to normal patients, or those with renal insufficiency, azotemia, hyperphosphatemia, and acidosis may occur. He suggested that tetracyclines exert their effect by inhibiting amino acid incorporation into proteins. This decreased utilization of amino acids results in an increased load of the products of amino acid catabolism presented to the kidney.

Nephrogenic diabetes insipidus after tetracycline usuage has also been described (9,10). Impairment of urinary concentrating ability was seen after protracted administration of therapeutic doses of nondegraded dimethylchlortetracycline hydrochloride. This seems to be totally reversible within four weeks of drug discontinuation. Polyuria and hypokalemia have also been described (11). Hypokalemic nephropathy was suggested as a possible cause of the polyuria which was corrected after potassium levels were brought to normal limits. Kuzucu (12) pointed out that tetracyclines given at the time of methyoxyfluorane anesthesia will produce deposition of calcium oxalate crystals and renal failure. The mechanism of this additive or synergistic effect of these two agents on the kidney was not elucidated. Tapp and Lowe (13) showed that tetracycline induces tubular necrosis in rats' kidneys if hemoglobinuria is present, and suggested that this might be due either to a short period of ischemia produced by the tetracyclines or to a direct nephrotoxic effect of tetracyclines. The nephrotoxic effect of large intravenous doses of tetracycline given to rats was shown to be prevented by concomitant administration of large doses of ascorbic acid (14). These protective effects were postulated to be secondary to an osmotic diuretic effect as well as a decreased protein binding in the plasma by ascorbic acid.

Hepatic toxicity has also been well documented. The hepatic lesions seen after tetracycline administration are rare in children. However, Dowling and Lepper (15) reported a case of a four-year-old boy who was given large doses of tetracycline and sulfisoxazole for treatment of pneumococcal meningitis, and at autopsy fatty metamorphosis of the liver was noted. Much of the earlier work on fatty liver changes associated with tetracycline was reported in pregnant females given intravenous tetracycline (16,17). However, other cases have been reported in nonpregnant females (18), in castrate male patients (19), and in adult males (20). These patients had characteristic liver changes specifically involving the central and midzonal portions of the liver lobule, with fine fat vacuolization giving the cells a foamy appearance.

Acute pancreatitis has also been documented after the use of tetracycline (20,21). This was seen in third trimester pregnant females who were treated with large intravenous or oral doses of tetracycline for pyelonephritis. An elevated serum amylase was also seen in a nonpregnant female post cholecystectomy and sphincterotomy after being given intravenous tetracycline (22). This elevation might have been due to the manipulation of the pancreas when performing the sphincterotomy. Focal hemorrhagic pancreatitis was also reported in a 72-year-old man given intravenous tetracycline for treatment of cystitis and diverticulitis (23). Impaired glucose tolerance was not reported in any of these cases.

The effects of tetracyclines on the hematopoietic system include the following: leukocytosis, atypical lymphocytes, toxic granulation of granulocytes, and thrombocytopenic purpura (24). Takahashi et al (25) reported a case of a 25-year-old female with tetracycline-induced Coombs positive hemolytic anemia. The mechanisms for this hemolytic anemia were believed due to a direct toxic action of the drug on the red blood cell, a hypersensitivity to the drug, or hemolysis due to drug effect on a congential enzymatic abnormality of the red blood cell. Lalezari et al (26) also cited two cases of immune hemolytic anemia occurring following the administration of tetracycline. In one of these cases, hemolysis occurred after two days of exposure to the drug which suggested an anamnestic type of response.

In view of the multisystem involvement in this case, it is important to cite the cases reported by Domz (27). He described de novo appearance of systemic lupus erythematosus, exacerbations of known lupus erythematosus (LE) and positive LE tests without clinical signs or symptoms of LE in patients treated with tetracyclines.

The only neurologic effect of this drug reported in the literature is pseudotumor cerebri seen in children. The syndrome is characterized by elevated intracranial pressure without focal neurologic signs, convulsions, or changes in mentation, and with normal or diffuse mild changes on the EEG (28,29).

The patient's initial complaints of nausea, anorexia, and frequent bowel movements were most likely secondary to gastrointestinal irritation by oral tetracycline or to progressive renal disease. These symptoms abated after discontinuation of the drug.

The abnormal EEG pattern recorded on admission may have been related to a toxic metabolic effect of tetracycline since marked improvement was seen after cessation of the drug. The age of this patient and the clinical presentation was not characteristic of pseudotumor cerebri.

The patient had a normally functioning exocrine pancreas and no signs of acute pancreatitis. However, the onset of diabetes mellitus may have been related to tetracycline although the diabetes persisted after withdrawal of the drug. The hepatorenal abnormalities and hemolytic anemia disappeared after discontinuing tetracycline.

This case emphasizes the importance of careful evaluation of patients before tetracycline is given as well as the dangers of its indiscriminate and prolonged use.

Acknowledgments

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Atypical Small-Cell Dysplasia and in Situ Carcinoma of the Breast: An Analysis of Subsequently Developing Ipsilateral Invasive Carcinomas

CYRIL TOKER, M.D.

Abstract

Thirteen invasive carcinomas developing in patients with previously demonstrated atypical small-cell proliferations of the mammary ducts and lobules are analyzed. Five of these subsequently appearing ipsilateral invasive carcinomas were small-cell tumors, 5 were large-cell tumors, and 3 were of mixed composition—containing both small- and large-cell populations. Some of the large cells may be derived from preexisting small cells, but it is possible that the majority of large cells may represent entirely independent lineages. Proliferation of the small cells may precede, by many years, the appearance of an invasive large-cell tumor. In approximately one half of the cases in which invasive tumors develop, however, the atypical small-cell proliferation will proceed to an invasive small-cell carcinoma.

The ensuing report will analyze the pathologic features of ipsilateral invasive carcinomas developing in patients with atypical small-cell dysplasia and in situ carcinoma of the mammary ducts and lobules (1,2). The relationship between the initially detected small-cell changes and the subsequent invasive tumors will be evaluated.

Material

A review of 4025 breast biopsies performed during the period 1946–1974 (inclusive) revealed 188 cases (4.6%) in which dysplastic lesions of solid, small-cell type (1,2) were present. The biopsies were mainly performed for fibrocytic disease, but breast tissue removed during mammoplasty, and breast tissue submitted together with fibroadenomas, have been included.

Pathology of the Initial Small-Cell Lesions

Two broad categories of small cell may be identified. The first—'myoepithelial'—category presents hyperchromatic eccentric nuclei (Fig. 1 and 2) and a moderate amount of eosinophilic cytoplasm. The cell membranes are usually clearly discernible, and the cells often occupy separate lacunae in the basal layer. These cells have a distinctly myoid appearance. The second—'syncitial'—category (Fig. 3) present central nuclei, either vesicular or hyperchromatic, surrounded by scanty cytoplasm. Cell membranes are not discernible, the cells growing in sheets. Admixtures of these small-cell types may be seen.

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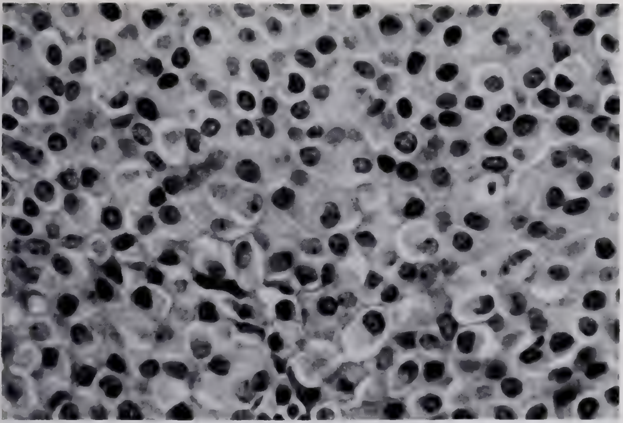

FIG. 1. Small cells of 'myoepithelial' type. Nuclei are hyperchromatic and eccentric. Cytoplasm is abundant and cell boundaries are clearly visible (H&E × 320).

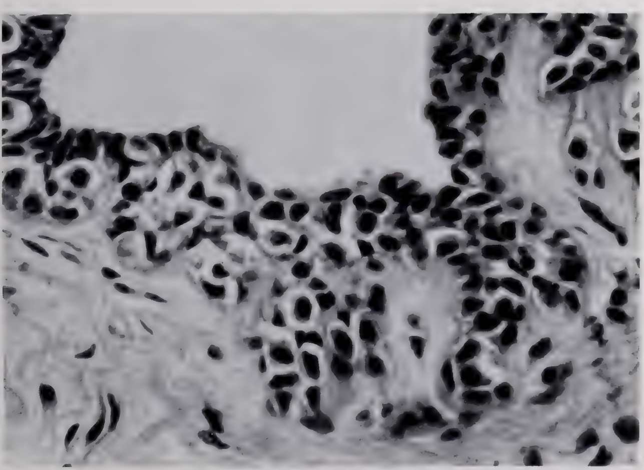

FIG. 2. Early atypical dysplasia (Grade I). The basally located 'myoepithelial' cells occupy separate lacunae (H&E × 320).

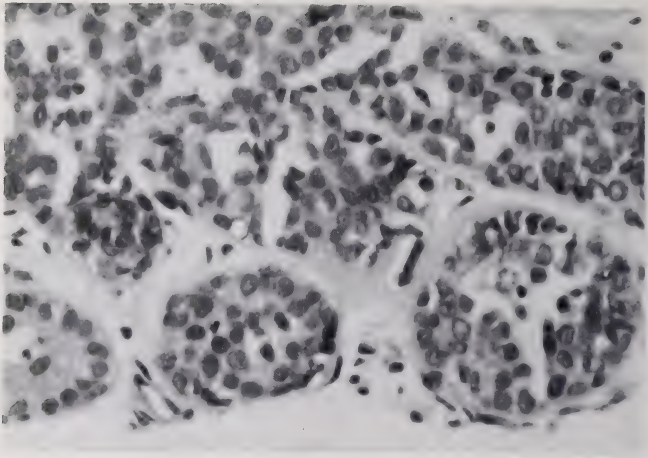

FIG. 3. Small-cell dysplasia, 'syncitial' type. The cells form sheets in which individual cell boundaries are not discernible (H&E × 320).

It should be noted that the small-cell category includes a range of sizes, with nuclei varying between some 8 and 15 microns. However, even the larger members of the small-cell group (Fig. 4) display a characteristic uniformity of configuration, the cells being polygonal or spherical. The pleiomorphism of large-cell proliferations (Fig. 5) is lacking, both with regard to nuclear and cytoplasmic outline. Mitotic activity, although occasionally present, is not a feature of small-cell proliferations.

The possibility that small cells may undergo enlargement to a large-cell form is suggested by appearances such as that depicted in Figure 6. Large cells with giant nuclei and abundant cytoplasm are closely integrated into the small-cell masses. No independent large-cell tumor was present in this instance, and the phenomenon is not explicable on the basis of collision.

Small-cell proliferations have been divided into 3 groups, (1,2), according to the degree of proliferation—Grade I (dysplasia), Grade II (indeterminate), and Grade III (frank in situ carcinoma).

Pathology of Subsequently Developing Invasive Carcinomas

To date, 13 infiltrating carcinomas have developed in patients available for follow-up (Table I). Four of these invasive tumors developed during the 5-year period follow biopsy, 8 during the 6- 15-year period subsequent to biopsy, and 1 after an interval of 18 years.

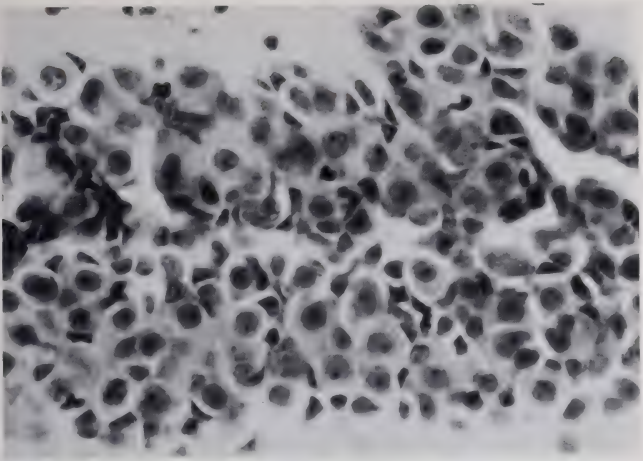

FIG. 4. Small cells of larger size than illustrated in the preceding micrographs still display uniformity of nuclear and cytoplasmic configuration (H&E × 210).

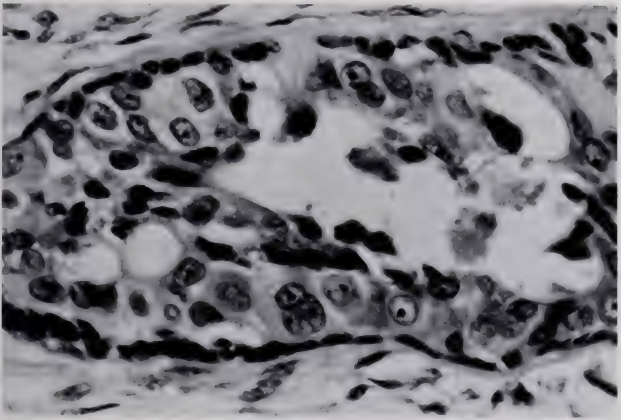

FIG. 5. Intraductal large-cell proliferation. Giant nuclei and cellular pleiomorphism are evident (H&E × 320).

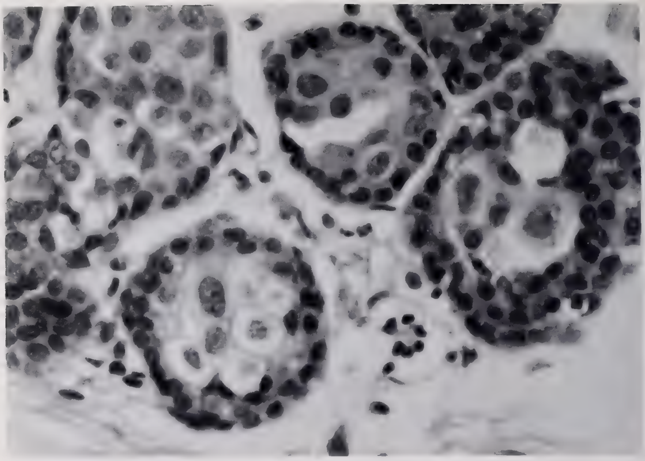

FIG. 6. Small-cell proliferation containing large cells closely integrated into the small-cell masses. No independent large-cell lesion was present, and it is possible that the large cells may have resulted from enlargement of the small cells (H&E × 320).

TABLE I

Invasive Carcinomas Subsequent to Detection of Small-Cell Cysplasia or in Situ Carcinoma on Previous Biopsy

Type of Invasive Tumor	Number of Cases
Small-cell	5
Large-cell	5
Mixed (small- and large-cell)	3

These subsequently appearing invasive cancers presented a varying morphology. Five lesions were infiltrating small-cell tumors. In this group the infiltrating cells maintain an appearance similar to that within the original in situ small-cell dysplasia or carcinoma (Fig. 7). The cells remain small, although some variation in size and some enlargement, may be observed. Five lesions were invasive large-cell tumors (Fig. 8), in which an invasive small-cell component was lacking. However, even in this group, residual in situ small-cell proliferations were found in addition to the invasive large-cell tumor. The 3 remaining infiltrating tumors exhibited mixed populations in which both small and large cells were present. In some areas large cells were closely integrated into the small-cell masses, presenting appearances similar to that illustrated in Figure 6; in others the small- and large-cell populations were quite separate.

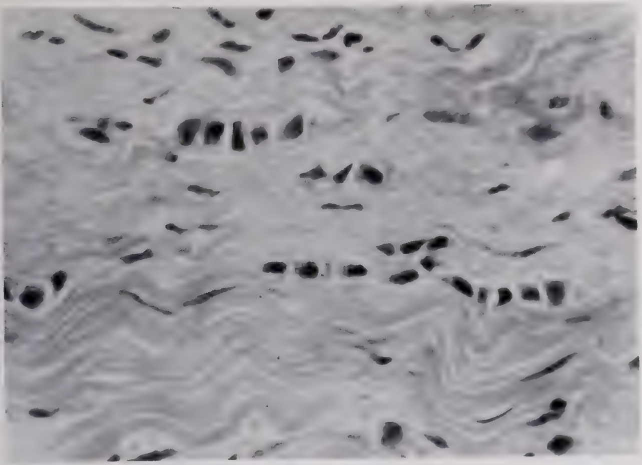

FIG. 7. Infiltrating small-cell carcinoma (H&E × 320).

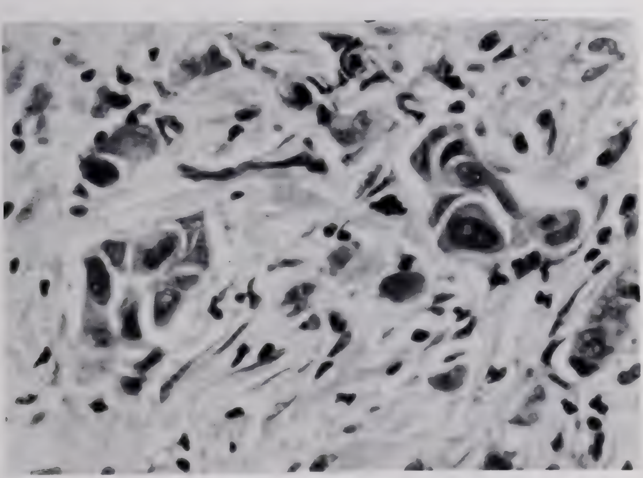

FIG. 8. Infiltrating large-cell carcinoma. Cell size and cellular pleiomorphism contrast with the features illustrated in the preceding micrograph of an invasive small-cell carcinoma (H&E × 320).

Comment

Atypical small cell dysplasias and in situ carcinomas have proceeded to invasive small-cell carcinomas in 5 out of 13 instances in which invasion supervened. Within another 5 patients the subsequently occurring invasive tumors were of large-cell type. In 3 patients the subsequent invasive cancers contained both small and large-cell populations.

The association of small-cell lesions with large-cell invasive cancers is apparent in the everyday examination of breasts resected for invasive tumors. Such examinations do not, however, provide any index of the temporal relationship between these entities, or of the sequences involved. Follow-up studies alone illustrate the long period—as long as 18 years on one occasion—that may elapse between the initial detection of the small-cell change and the later development of an invasive large-cell tumor. Morphologic observations suggest that some of the subsequently appearing large cells may have resulted from enlargement of preexisting small cells; but the large- and small-cell lineages may well be entirely independent on the majority of occasions. Under these circumstances we may postulate the action of different carcinogens at different times on different segments of the mammary epithelium. If, alternatively, the same agents are constantly at work, varying periods of exposure may be required before their effects are manifest among different cell populations. It may be noted that large-cell activity was not observed, in the initial biopsy, in any of the patients who subsequently developed large cell invasive tumors. We have, however, observed coexistent independent in situ small- and large-cell proliferations in breast biopsies, indicating that these processes may, on occasion, occur simultaneously.

The invasive potential of the in situ small-cell lesion is evident. It is, however, impossible to predict the cytology of a subsequently developing invasive tumor in every patient with an atypical small-cell proliferation. On some occasions the latter will appear to be no more than a barometer heralding the action of carcinogenic stimuli which will, at a later stage, produce an invasive cancer of quite a different type.

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Pelvic Laparoscopy at The Mount Sinai Hospital: A Report of 384 Consecutive Cases at The Mount Sinai Medical Center, 1973–1974

A. CASTRO-MARIN, M.D., R. NACHAMIE, M.D., AND S. B. GUSBERG, M.D.

Laparoscopy was widely used in our department as an adjunctive diagnostic and therapeutic procedure in 1973. Between January 1973 and December 1974, a total of 384 laparoscopies were performed at The Mount Sinai Hospital. Of these, 96 were diagnostic procedures while 193 were primarily for the purpose of sterilization. Of the total number, approximately 70% were performed by the resident staff. All were inpatient procedures, under general anesthesia and using the double puncture technique. Complications included 2 small bowel injuries which occurred while the technique was still being developed and modified. During the last months complications have been few and the procedure is now an integral part of diagnostic investigation and treatment of several pelvic conditions.

Materials and methods: All patients who underwent elective laparoscopy were admitted on the evening prior to the procedure. They fasted from midnight to provide safe, general endotracheal anesthesia with assisted ventilation. Contraindications (Table IA) were limited to: previous extensive surgery, hernias, inability to tolerate anesthesia, chronic medical disease, generalized peritonitis, and marked obesity. Indications (Table IB) were: bilateral tubal fulguration, infertility investigation, diagnostic evaluation of pain or mass and localization and removal of translocated IUD's.

Tubal sterilization was the indication for which the procedure was most frequently performed. This is partly because of the public's increasing desire for permanent surgical sterilization and the financial and social benefits derived from the shortened hospitalization with the laparoscopic approach.

Infertility was another important indication for laparoscopy. In patients in whom no causes for sterility had been discovered, evaluation of the pelvic organs revealed in some cases previously unsuspected pathology such as: endometriosis, pelvic adhesions, and uterine or adnexal abnormalities. In addition, tubal insufflation and dye studies under direct visualization are increasingly replacing x-ray hysterography for evaluation of tubal patency and function.

Laparoscopy has proven valuable as a diagnostic tool in the early acute abdomen with sudden onset of lower quadrant pain with or without nausea, vomiting, fever, leukocytosis, or elevation of erythrocyte sedimentation rate.

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TABLE IA

1973–1974

The Mount Sinai Hospital Contraindications for Laparoscopy

- Previous extensive surgery
- Hernias
- Inability to tolerate anesthesia
- Chronic medical factors
- Generalized peritonitis
- Marked Obesity

TABLE IB

The Mount Sinai Hospital Indications for Laparoscopy
1973–1974

Bilateral tubal fulguration	183
Infertility	102
Diagnostic (pelvic pain and/or mass)	96
IUD localization	3
Total	384

Similarly, laparoscopy has been found valuable in defining the otherwise obscure cause of persistent chronic pelvic pain.

Technique: All patients are placed in modified dorsal lithotomy position with the legs flexed at their knees and in moderate abduction. The ground plate is placed if necessary under the patient, and skin preparation of abdomen, perineum and vagina is performed with betadine solution. The patient is draped properly, bladder is drained, cervix visualized and the vacuum cup device designed by Semm, or the Hulka tenaculum, is applied to the cervix for uterine manipulation. With the patient in deep Trendelenburg position, 2–4 liters of CO_2 are introduced into the peritoneal cavity by using the Veress cannula in the infra-umbilical region. When the pneumoperitoneum is obtained, a trocar followed by the laparoscope is introduced into the peritoneal cavity and immediate examination of pelvic and abdominal organs is performed. When desired, as with our double puncture technique, the second puncture site is made under direct visualization either in the midline supravesically or in the right lower quadrant. The examining probe and operating instruments are manipulated through this second puncture site.

Results: The findings at laparoscopy are listed in Table II. In 48 patients, no abnormalities were found. The positive and differential findings included: adnexitis, ovarian cysts, endometriosis, unruptured ectopics, fibroids, cancer of the cecum, and ruptured ectopics. The complications at laparoscopy are listed in Table III; hemorrhage was the most common complication occurring in 15 cases. This included bleeding in the mesosalpinx, infundibulo-pelvic ligament, and broad ligament. Bowel injury occurred in two patients and electrical burn of the abdominal wall in 1. Uterine perforation was detected in 2 patients and pregnancy occurred following two instances of tubal fulguration. In one case the patient was pregnant preoperatively and in the second case the round ligaments had been cauterized. Cardiac arrhythmia occurred in 1 case and wound infection in 2 cases for a total of 25 complications. There were 11 cases of unsuccessful laparoscopies. The causes for failure are listed in Table IV. They include CO_2 leak, poor visualization, and inability to enter the peritoneal cavity.

TABLE II

The Mount Sinai Hospital Findings at Laparoscopy
1973–1974

Adnexitis	49
Ovarian cyst	21
Endometriosis	19
Unruptured ectopics	13
Fibroids	9
Cancer of cecum	1
Ruptured ectopics	3
Total	115
No Abnormal Findings	48

TABLE III

The Mount Sinai Hospital Complications
1973–1974

1. Hemorrhage (mesosalpinx, infundibulo-pelvic, broad ligament)	15
2. Bowel injury	2
3. Electrical burn of abdominal wall	1
4. Uterine perforation	2
5. Pregnancy	2
6. Cardiac arrhythmia	1
7. Wound infection	2
Total	25

TABLE IV

The Mount Sinai Hospital Failed Laparoscopies

Number	11
Causes:	
1. CO_2 leak	
2. Poor visualization	
3. Unable to enter peritoneal cavity	

Discussion

The value of laparoscopy is evident as a diagnostic and therapeutic adjunct. Although laparoscopy can never replace clinical judgment and diagnostic expertise, laparoscopy is an extension of the diagnostic armamentarium that can enrich the objective data base. It has replaced culdoscopy as a primary endoscopic procedure. The rapid recovery following diagnostic and surgical laparoscopic procedures provides shorter hospital stay with subsequent saving to the patient and hospital and quicker return of the patient to her home, family and daily living.

We conclude with others, that careful selection and preparation of the patients with methodical execution of the procedure can significantly limit the complications of laparoscopy. We emphasize the importance of an inpatient procedure with general endotracheal anesthesia and all of the precautions attendant upon general anesthesia. The equipment should be understood by all who use it and maintained in good functioning order. Satisfactory pneumoperitoneum must be maintained for good visualization. When the procedure is carried out under carefully executed conditions both the patient and physician can benefit.

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Neurogenic Disorders Affecting Muscle: Five-Year Analysis of Muscle Biopsies

II. Myopathic Changes

SEYMOUR GENDELMAN, M.D., SUN K. SONG, M.D., AND PAUL J. ANDERSON, M.D.

The tissue diagnosis of neurogenic atrophy has long been based on the histologic features of segmental atrophy (1-4). Skeletal muscle biopsy interpretation was based on the observation that following experimental denervation, the muscle fibers appropriate to the affected motor unit showed atrophy without structural change (5). Occasional reports have suggested that some of the observed structural changes indicated an associated myopathy (5-7). However in recent years a number of reports have appeared showing that previously considered myopathic changes have not been limited to muscle diseases wholly of myopathic origin (8, 9).

As part of a five-year prospective study of muscle biopsies (10), the presence of structural changes in patients with known neurogenic disease was investigated.

Materials and Methods

172 patients with clinical evidence of neurogenic disease had 265 muscle biopsies over a five-year period.

Clinical assessment of patients preceded and was independent of biopsy analysis. The patients were divided into 3 clinical groups: myelopathy (78 cases); peripheral neuropathy (85 cases); and a mixed group (9 cases). The criteria for clinical diagnosis is described elsewhere in detail (11-15). Most of the patients clinically diagnosed as myelopathy had findings compatible with diffuse involvement of the spinal cord, such as amyotrophic lateral sclerosis and progressive spinal muscular atrophy. All patients with the clinical diagnosis of peripheral neuropathy had polyneuropathies. Polyneuropathies were not clinically differentiated from polyradiculopathies and both were considered as part of the neuropathy group unless associated evidence for spinal cord disease was also present. The mixed group contained clinical features of both the spinal cord and the peripheral groups. The various associated illnesses included diabetes mellitus, alcoholism and nutritional deficiencies, infections, collagen diseases, or other systemic disease.

The histologic features were systematically recorded without regard to the clinical diagnostic impression. Methods for plotting muscle fiber size have been previously reported (16).

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Biopsies from 137 patients with varying myopathic disorders were available for comparative analysis. The diseases included progressive muscular dystrophy, polymyositis, endocrine myopathy, and other myopathies of unknown etiology. Laboratory studies included electromyography, serum enzymes, blood chemistries, urinary excretion of creatine, creatinine, hematologic and endocrine studies, and appropriate x-ray studies.

Results

1. Sarcoplasmic changes (Figs. 1 and 2): Table I shows the major sarcoplasmic changes encountered in biopsies from patients with neurogenic disorders of muscle. Of the 101 cases that showed sarcoplasmic alterations, 98 cases included one or more of these changes. The most frequently seen sarcoplasmic alteration was floccular change, encountered in 62 patients (35%). Less frequently seen were phagocytosis in 53 patients (30.8%), basophilia in 44 patients (25.6%), and target or targetoid fibers in 24 patients (14%). Forty patients (23.3%) showed inflammatory change to varying degrees.

Comparison of the neurogenic and myopathic populations showed that these sarcoplasmic alterations were generally more common in the myopathic group (Table II).

Target or targetoid fibers were more frequent in the neurogenic group but also were encountered in a small number of patients with myopathic disorders.

2. Muscle fiber form (Table III): Angulated fibers were more frequently encountered in the neuropathic group, while rounded fibers occurred more frequently in the myopathic group. Ring forms were somewhat more evenly distributed. Angulated, rounded, and ring fibers were studied in cross section, and their incidence in the neurogenic group compared with that in the myopathic group previously described.

Discussion

It was generally assumed that sarcoplasmic changes are not a frequent occurrence, and certainly not the predominant histologic feature of neurogenic

TABLE I

Sarcoplasmic Changes—Relationship of clinical groups to types of sarcoplasmic change: floccular change, phagocytosis, basophilia, target fibers, and inflammation.

	Cases	Floccular change	Phagocytosis	Basophilia	Inflammation	Target Fibers
Myelopathy 78 cases	37 (47.4%)	25 (32.1%)	21 (26.9%)	21 (26.9%)	14 (17.9%)	5 (6.4%)
Neuropathy 85 cases	58 (68.2%)	33 (38.9%)	30 (35.3%)	20 (23.5%)	22 (25.9%)	16 (18.8%)
Mixed 9 cases	6 (66.7%)	4 (44.4%)	2 (22.2%)	3 (33.3%)	4 (44.4%)	3 (33.3%)
Total 172 cases	101 (58.8%)	62 (36.0%)	53 (30.8%)	44 (25.6%)	40 (23.3%)	24 (14.0%)

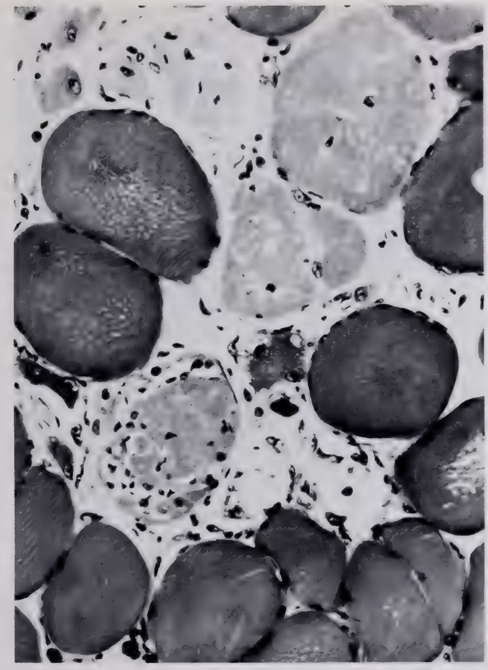

FIG. 1. Sarcoplasmic structural change; muscle fibers showing floccular change and phagocytosis, approximately 180×.

muscular disease. Greenfield et al noted sarcoplasmic changes in biopsies from four of nine patients with amyotrophic lateral sclerosis, but felt that these changes suggested a separate "myopathic" component (3).

This suggestion was followed by popular usage of biopsy terminology: "Mixture of myopathic and neuropathic features" (6).

Examination of the sarcoplasmic structural changes encountered in the present study showed that there was a greater incidence of these changes than originally anticipated. Almost 60% of biopsies from patients with neurogenic disorders showed some degree of sarcoplasmic structural alteration. The major areas of change were of five types—floccular change, phagocytosis, basophilia, inflammation, and target or targetoid fibers. Only the distribution of target fibers differed markedly from the other changes, occurring more frequently in the neurogenic than the myopathic group.

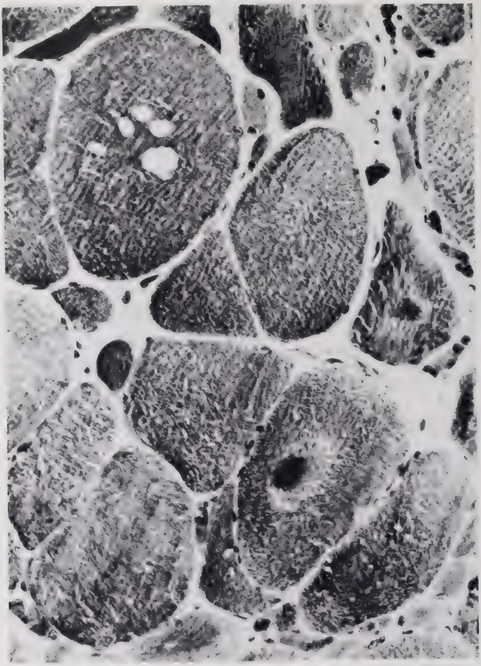

FIG. 2. Sarcoplasmic structural change; muscle fibers showing target and targetoid change in the presence of "segmental atrophy," approximately 240×.

TABLE II

Sarcoplasmic Change—Sarcoplasmic Changes in Neruogenic and Myopathic Disease. With the exception of target fibers, all changes had a greater incidence in the myopathic group.

	Cases	Floccular change	Phagocytosis	Basophilia	Inflammation	Target fibers
Neurogenic	101	62	53	44	40	24
172 cases	(58.8%)	(36.0%)	(30.8%)	(25.6%)	(23.3%)	(14.0%)
Myopathic	98	73	68	64	53	5
137 cases	(71.5%)	(53.3%)	(49.6%)	(46.7%)	(38.7%)	(3.6%)

An incidence of sarcoplasmic alteration in 58% of muscles in neurogenic disorders cannot be due to chance alone, nor could such a degree be due to unrelated association of neuropathy and myopathy. Following simple nerve section, structural alterations of this sort are uncommon, though in other

TABLE III

Abnormal Fiber Forms—The occurrence of the various abnormal fiber forms in all cases of neurogenic atrophy and a diverse myopathic group. Only in the neurogenic group, was there a greater incidence of angulated fibers.

Form	Neurogenic	Myopathic
Cases	172	137
Angulated	82 (48.2%)	51 (37.2%)
Rounded	76 (44.2%)	73 (53.3%)
Ring Forms	25 (14.5%)	28 (20.4%)

types of experimental injury, muscle fibers may show some of these changes (17). Electron microscopy of experimental denervation showed a loss of myofilaments, reduction in volume of myofibrils, loss of sarcomeres, and alteration of the sarcoplasmic reticulum (18). However, these changes appear to differ somewhat from those generally seen in the myopathies.

It seems that the degree of sarcoplasmic alteration encountered is more probably related to whatever the primary process may be, in this instance, a neurogenic disorder of muscle. The classic concept of neurogenic disease of muscle requires reevaluation in view of these more recent developments.

The incidence of various fiber forms was not significantly different in either category, and their presence was frequent in both groups.

Conclusions

There was a significant incidence of sarcoplasmic structural change in neurogenic disorders of muscle. These sarcoplasmic alterations were most commonly of 5 types: floccular change, phagocytosis, basophilia, inflammation, and target for targetoid fibers. Similarly alterations of muscle fiber form were frequently present in both groups.

The diagnosis of "neurogenic atrophy" does not preclude the presence of sarcoplasmic structural change in muscle. It is suggested that sarcoplasmic changes encountered in neurogenic disorders be accepted as an integral part of the pathologic process, and not be given independent "myopathic" status.

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Composite Tumor of Small Intestine*

STEVEN L. GOLDBERG, M.D. AND CYRIL TOKER, M.D.

Abstract

A composite tumor of the small intestine, containing both adenocarcinoma and carcinoid, is reported. This lends further support to the contention that at least some cells of the argentaffin cell system (argentaffin, argyrophil, and clear cells) are of endodermal origin and are developmentally related to other specialized mucosal cells (goblet, Paneth, and parietal cells). Similar tumors have previously been recorded at other sites within the gastrointestinal tract, and it is predicted that similar neoplasms, possibly associated with the carcinoid syndrome, will be encountered within other organs containing both exocrine and endocrine components.

Introduction

Small intestinal carcinomas constitute 1% of gastrointestinal carcinomas, and small intestinal carcinoids constitute 28% of gastrointestinal carcinoids (1). In most series of small bowel tumors, carcinomas occur two to three times as frequently as carcinoids, but occasionally the reverse is reported (2). Their occurrence together as separate tumors in the small intestine has been reported (3). The present case documents the occurrence of both neoplastic elements in the same tumor at this site.

Case Report

A 40-year-old Caucasian male had a two-week history of periumbilical pain associated with anorexia, weight loss, and one episode of rectal bleeding. Barium enema was normal. Small bowel series showed an ulcerating mass in the proximal jejunum. The tumor was removed, and no other lesion was found on examination of the abdominal contents.

Pathologic Findings: The specimen was sent directly to the laboratory for frozen section and immediately fixed in 10% formalin. It consisted of a 15 cm segment of jejunum with a 5 x 4 x 2.5 cm, ulcerated, firm, nodular mass extending through the entire wall. Serosal involvement was extensive.

Histologic examination of the tumor revealed a well-differentiated adenocarcinoma involving the mucosa. The tumor extended through the entire bowel wall. In several areas the neoplastic glands were continuous with solid cords, rests, and sheets of small, round, uniform cells (Figs. 1, 2). Argentaffin, argyrophilic, and ultraviolet autofluorescent granules were not demonstrated in any of the tumor cells.

Discussion

The composite tumor (composite carcinoid tumor, argentaffinoma-adenocarcinoma) containing adenocarcinoma and carcinoid is a well-estab-

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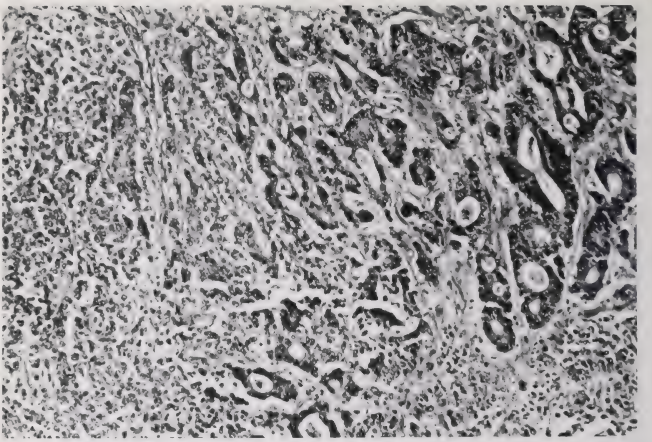

FIG. 1. Micrograph showing well-differentiated adenocarcinoma (right side of figure) intermixed with carcinoidal trabeculae (left side of figure) (H & E × 80).

lished entity. It has been noted in the colon, appendix, stomach, and gallbladder (4–12). The present case report describes such a tumor in the small intestine.

The intimate association of the dual elements argues against a collision tumor. We believe, as previously (7), that the trabecular areas represent carcinoid differentiation and not merely anaplasia within the carcinoma. The demonstration of specific granules varies in different carcinoid tumors even under optimum conditions of fixation, and though small intestinal carcinoids characteristically contain granules, this is not invariable (13–16).

The relationship between the argentaffin system (argentaffin, argyrophil, and clear cells), the precursors of the carcinoid tumors, and the other specialized mucosal cells of endodermal origin (goblet, Paneth, and parietal cells), the precursors of the adenocarcinomas, is an intimate one. They may be derived from a common precursor cell and/or from each other, but in either case at least some of the argentaffin (Kultschitzky) cells are believed to have a mucosal, endodermal origin. If this supposition is not correct, it must be presumed that argentaffin cells of neural crest origin are capable of differentiating into mucin-secreting mucosal cells. Argentaffin cells appear early in the embryonic gut even before the mucin-secreting cells, and argentaffin cells appear or increase in number when there is hyperplasia of endodermally derived mucosa, as in chronic inflammation, or in mucosa adjacent to carcinomas (17–19). Further examples of the sharing of common developmental properties can be seen in mucin production by carcinoid tumors and

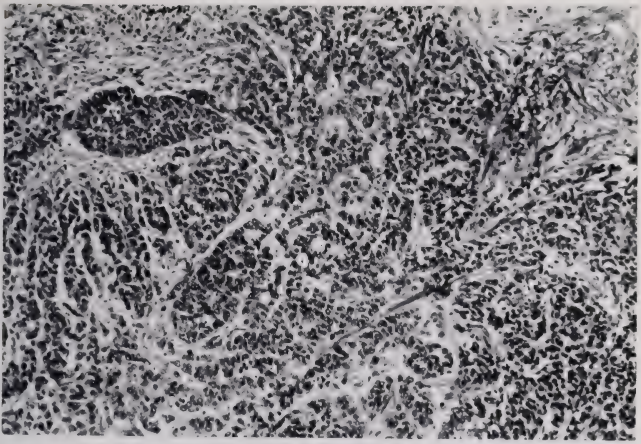

FIG. 2. Micrograph showing carcinoidal trabeculae (H & E × 80).

the presence of argentaffin granules in some adenocarcinomas (13, 20, 21). Indeed, a carcinoid tumor was observed to assume the adenocarcinoma pattern on heterotransplantation (22). It is, therefore, not surprising that a mucosa capable of differentiating into multiple cell types normally is also capable of producing a tumor displaying more than one histologic pattern derived from those cell types.

As previously predicted, composite tumor may be anticipated within other organs containing both exocrine and endocrine components (7). These will include lung, prostate, urinary bladder, kidney, ovary and pancreas. Since the carcinoid component of composite tumors may be granule-containing, it can further be predicted that the carcinoid syndrome will be encountered with such tumors.

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Pseudolymphomatous Pulmonary Infiltrates in Rheumatoid Arthritis

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Abstract

A patient with classic rheumatoid arthritis had myocardial rheumatoid nodules and localized pseudolymphomatous pulmonary infiltrates. He had prostatic carcinoma and died from massive hemorrhage caused by a clinically unsuspected gastric carcinoma. The possible pathogenesis of the pulmonary infiltrate is postulated.

Rheumatoid arthritis may be a systemic disease involving many organ systems (1–2). The extra-articular organs commonly involved are the heart and lung. Granulomatous nodules of the cardiac valves and myocardium, although infrequent, have been well described (3–5). No specific and characteristic lung lesions have been associated with rheumatoid arthritis (6), although a wide range of pulmonary lesions including pleuritis with or without effusion, interstitial pneumonitis, and granulomatous nodules have been observed (7–11). This report describes a patient with rheumatoid arthritis who had rheumatoid nodules in the myocardium and localized pseudolymphomatous pulmonary infiltrates. This type of pulmonary infiltrate has not been frequently recorded in patients with rheumatoid arthritis.

Case Report

A 68-year-old Puerto Rican man was admitted to The Mount Sinai Hospital for the seventh time in November 1971, for progressive lethargy and fecal and urinary incontinence. At admission, he was in shock and died during the first hospital day.

Physical findings included flexion deformity of elbows and wrists, digital shortening and ulnar deviation of the hands, smooth and atrophic skin over the affected joints, a grade III/VI holosystolic murmur, and an enlarged, firm, nontender prostatic gland.

He was first seen in August 1966. He had had severe arthritis for more than 10 years and the latex fixation titer was 1:2560. He developed wheezing and exertional dyspnea and was treated with digitalis, diuretics, and sodium restriction.

In December 1966, he was admitted for hematuria and prostatic hypertrophy. Transurethral prostatic resection was performed. Infiltrating prostatic adenocarcinoma was found, however, he refused orchiectomy.

During the next five years, multiple admissions were prompted by congestive heart failure, bronchopneumonia, and urinary tract infections. Because of increasing frequency and urgency of urination, he was started on Stilbestrol in June 1971.

During the previous admissions, a number of laboratory tests had been performed. The hemoglobin was 10 gm/100 ml with a hematocrit of 31% and an erythrocyte sedimentation rate of 100 mm. The total serum bilirubin determination was 3.5 mg/100 ml and the alkaline phosphatase was 160 U with a glutamic oxaloacetic transaminase (SGOT) of 52 U. The total serum protein was

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8.4 gm% with gammaglobulin of 4.74%. Serum immunoglobulin showed an IgG of 3400 mg/100 ml, IgA of 1450 mg/100 ml and IgM of 420 mg/100 ml. Cellulose acetate immunoelectrophoresis demonstrated a diffuse increase in all immunoglobulins without spikes. The Sia test was negative. Serum iron was 35 microgram/100 ml, unsaturated iron binding capacity was 230 microgram/100 ml and total iron binding capacity was 265 microgram/100 ml. Anti-nuclear antibodies were not detected and the LE preparation was negative. Titers of rheumatoid antibodies, determined by the latex fixation test, varied from 1:1280 to 1:5120. Serologic tests for syphilis (VDRL and Kahn) were weakly positive. A roentgenogram of the chest, two months before death, was clear without infiltration or masses and with blunting of right costophrenic angle from effusion. The titer of antibodies of the pleural fluid, as determined by the latex fixation test, was 1:1280.

Necropsy: At autopsy, severe deforming rheumatoid arthritis involving numerous joints was observed. There was severe ulnar deviation of hands and subluxation of finger joints. The stomach was dilated and contained about 2000 cc of fresh blood and blood clots. A 5 × 4 cm fungating adenocarcinoma was in the gastric cardia near the esophagogastric junction. The small bowel and colon contained approximately 1000 cc of blood clots. The perigastric lymph nodes were involved by metastatic gastric adenocarcinoma. The firm, yellow, nodular prostate was extensively infiltrated by well-differentiated prostatic adenocarcinoma. However, the seminal vesicles and adjacent lymph nodes were free of tumor.

The enlarged heart weighed 450 gm. The pericardial surfaces were covered by fibrin and hemorrhage and were adherent. The left ventricular myocardium was hypertrophied (1.8 cm). The leaflets of both the mitral and aortic valves were thickened and rigid with focal calcification. Several grey-white, well-circumscribed nodules, the largest approximately 0.8 cm in diameter, were in the posterior wall of the left ventricle (Fig. 1). Histologically, these nodules displayed the features of the classic rheumatoid granuloma with central fibrinoid necrosis surrounded by palisading fibroblasts, histiocytes, lymphocytes and plasma cells (Fig. 2). Small foci of ossification were observed in some of the nodules. The lungs weighed 1,100 gm together and there was a fibrous pleuritis. There were small focal areas of consolidation scattered throughout lung parenchyma. Histologically, the lung showed focal areas of edema and interstitial and intra-alveolar fibrosis. In some areas, lymphocytes and plasma cells were aggregated into small follicles, some

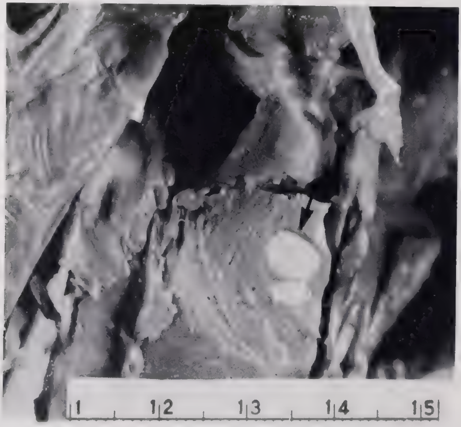

FIG. 1. Left ventricular myocardium showing rheumatoid nodules (arrow).

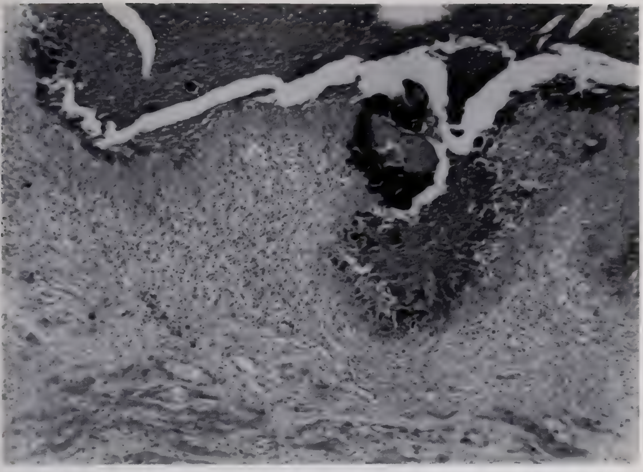

FIG. 2. Histologic appearance of part of rheumatoid granuloma with central fibrinoid necrosis (above) surrounded by palisading fibroblasts, histiocytes, lymphocytes, and plasma cells (HE × 40).

of which had germinal centers, forming pseudolymphomatous pulmonary infiltrates (Figs. 3 and 4). The pulmonary hilar lymph nodes had enlarged follicles with prominence of germinal centers. The enlarged spleen weighed 340 gm and had moderate reticuloendothelial hyperplasia and congestion.

Discussion

The patient described in this report had a long history of rheumatoid arthritis with clinical manifestations of valvular incompetence, frequent congestive heart failure, and recurrent respiratory infections. He was also known to have had well-differentiated prostatic adenocarcinoma, and he died from clinically unsuspected gastric adenocarcinoma with sudden massive hemorrhage.

Two primary carcinomas in this patient with rheumatoid arthritis may be coincidental. Although the association has been observed (7, 12), there is no convincing evidence of an increased incidence of malignant disease in patients with rheumatoid arthritis (13).

The pseudolymphomatous pulmonary infiltrate may be localized or diffuse and has been designated as lymphoid interstitial pneumonia (LIP) by Liebow (14). Histologically, many mature lymphocytes and plasma cells are present in the pulmonary interstitium or alveolar spaces. Germinal centers may be seen. The epithelium of distal air spaces is often entrapped in the dense lymphoid

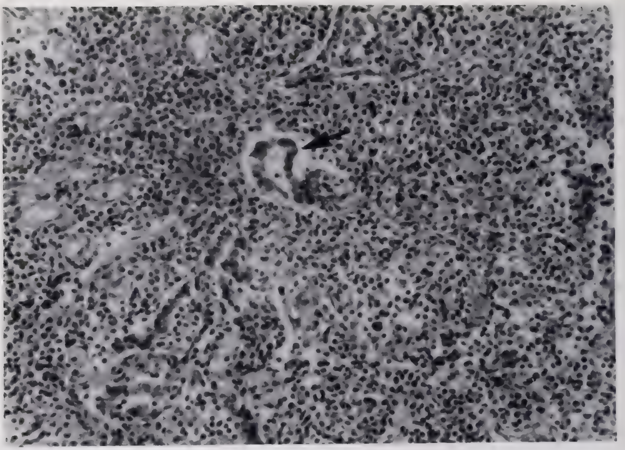

FIG. 3. Pulmonary interstitial and intraalveolar dense lymphoid infiltrate. Entrapped respiratory epithelium (arrow) can be seen (HE × 100).

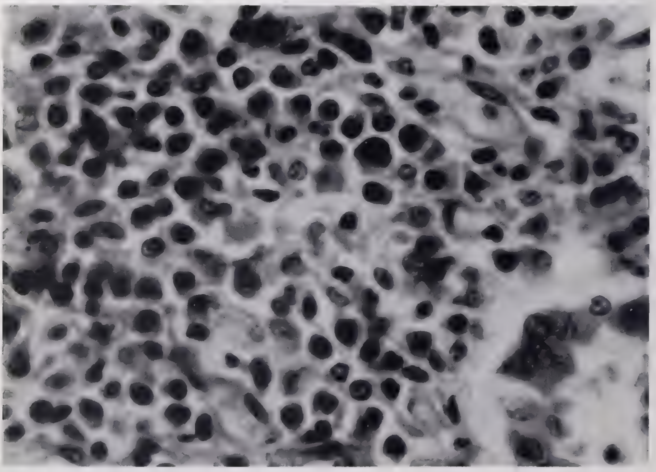

FIG. 4. High magnification of pulmonary infiltrate containing predominantly mature lymphocytes and plasma cells (HE × 100).

infiltrate, although there is no evidence of epithelial destruction. This condition has been described in recent years most commonly associated with Sjögren's syndrome or abnormalities in blood proteins (15–17). Rheumatoid arthritis is commonly associated with Sjögren's syndrome and often manifests hyperimmunoglobulinemia (18). It is not unreasonable, therefore, to expect that a pseudolymphomatous pulmonary infiltrate (LIP) may also be seen in patients with rheumatoid arthritis. However, this association has not been made. Reich et al (19) described an 11-year-old boy treated for "rheumatoid arthritis" after streptococcal throat infection. He had a right pleural effusion, increased serum immunoglobulin, and a pulmonary mass in the right middle lobe. This was resected and reported as benign lymphoid hyperplasia.

The pathogenesis of pseudolymphomatous pulmonary infiltrate in rheumatoid arthritis is not clear. The lung in this condition resembles the thyroid in Hashimoto's disease and the salivary gland in Mikulicz's syndrome. Because of this resemblance, it has been suggested that the condition might represent a phenomenon of autoimmune disease (14). Indeed, DeHoratius et al (18) demonstrated a large amount of IgM antiglobulin and a small amount of IgG antiglobulin in the pulmonary alveolar wall and capillary bed in "rheumatoid lung." The entrapment of immune complexes in lung tissue may be responsible for this unusual pulmonary infiltrate.

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Osteogenic Sarcoma following Radiotherapy for Bursitis

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Abstract

Twenty-three years following radiation treatment for bursitis, the patient reported here developed osteogenic sarcoma of the humerus and succumbed to that disease within six months. The records of the radiologist who gave the initial treatment were reviewed and it was found that the patient received no more than 800r using 200 KV x-ray. Even with this low dose, a radiation-induced fatality occurred. Since the evidence of any benefit from the use of radiation therapy in the treatment of bursitis is unconvincing in any event, there is no rationality in the continuation of this practice.

With our increasing knowledge of the late consequences of exposure to ionizing radiation, the use of radiotherapy in the management of benign conditions has virtually disappeared (1). For the most part, only a few pockets of "die-hards" remain and descriptions of the use of "malignant radiation for benign conditions" (2) are relevant to historical rather than current practice. Bursitis is one of the few areas where a tenuous veil of respectability may still cover the use of radiotherapy (3). The case reported here had a fatal outcome 23 years after a relatively small dose of x-ray (800 r) was delivered for bursitis.

Case Report

The patient was a 65-year-old female who in November 1971 developed pain in her right shoulder and was treated for subdeltoid bursitis by medical means. X-rays taken at her doctor's office were interpreted as normal, but x-rays two months later showed an osteolytic lesion in the proximal right humerus with pathologic fracture of the humeral head (Fig. 1). The patient was admitted to another hospital where a biopsy of the bone was taken and read as carcinoma, metastatic to bone. An extensive work-up was performed but no primary lesion was found. This included GI series, barium enema, chest x-rays, IVP, breast examination, including mammograms, pelvic examination, and pap smear. Review of the pathology at Mount Sinai suggested that the lesion was not metastatic carcinoma but a sarcoma, and further sections submitted revealed the presence of frank osteogenic sarcoma (Fig. 2). In addition, degenerative changes consistent with previous irradiation were noted within the bony trabeculae.

The patient remembered that in 1948 she had bursitis in this arm for which she received "some x-ray treatment." She remembered also that it did not improve and 3 months later she received another set of treatments. As far as the patient remembered, she had no further difficulty with this shoulder until the onset of pain in the current illness.

The original radiologist who gave the treatment for the bursitis in 1948 was located. He found the patient's records which revealed that he had used 200 KV x-ray with .5 mm Cu. added. In the first course of treatment, the patient received 5 sessions of 100 r each in air. Three months later he gave 3 additional sessions of 100 r. As far as the radiologist could ascertain, no further treatments were given to this patient.

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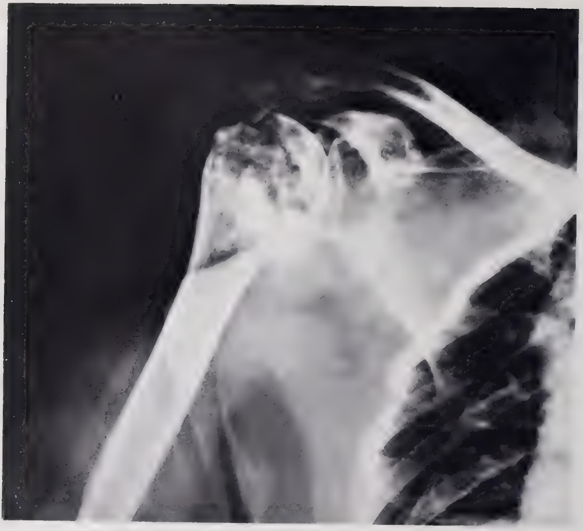

FIG. 1. Right shoulder showing osteolytic lesion with pathologic fracture of the head of the humerus.

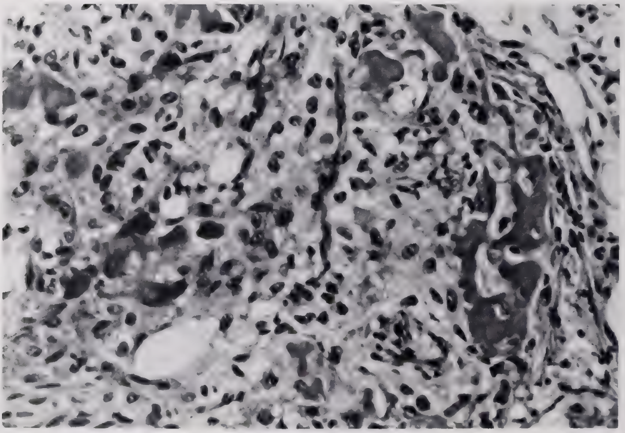

FIG. 2. Photomicrograph illustrating formation of osteoid by tumor cells (H & E × 200).

At Mount Sinai, in 1971, she received local radiation treatment to the right humerus for control of pain. Pulmonary metastases were seen in February 1972. The patient failed to respond to chemotherapy and expired in May 1972, six months following the initial treatment.

Discussion

The treatment of bursitis by radiotherapy became popular in the 1930's but as early as 1940 some skepticism about the effectiveness of the treatment had already been voiced by Newell (4). A clinical study of the value of x-ray therapy in calcifying tendinitis was reported by Plenk in 1952(5) which showed no value to this treatment. He concluded that the natural history for bursitis appears to be spontaneous improvement, including spontaneous disappearance of calcifications. Therefore the only treatment necessary is symptomatic relief during the acute phase. A much more sophisticated clinical trial reported in 1970 from Sweden (6), based on an experience with over 300 patients randomly assigned to irradiation or sham irradiation, resulted in no significant difference in subjective or objective response to irradiation.

On the other hand, exposure to irradiation is not without its risks. Beginning as early as 6 months after Roentgen's discovery, there already had accumulated considerable evidence of the deleterious biologic effects such as pigmentation, telangiectasis, fibrosis, scarring, alopecia, dermatitis, and ulceration. There also appeared a new cancer, man-produced with his newly created x-ray generators. Since 1902, a large number of papers have been published on radiation-induced neoplasm (7, 8).

Postradiation osteogenic sarcoma has been reported most frequently in patients treated for benign bone diseases, the first case having been recorded by Beck in 1922 (9). For the most part, the patients tend to have been young when irradiated and survived long enough for the radiation injury to become apparent (10–15). Another large number of cases in the literature occurred after the successful treatment of a malignancy. Postradiation osteogenic sarcomas have been reported in adults treated for cancer of the breast (10–18), cervix (18–21), bladder (13), seminoma (12–15), and lymphomas (12, 13, 15), and in children after treatment of retinoblastoma (10, 15, 22, 23), rhabdomyosarcoma (15), neuroblastoma (17), and Wilm's Tumor (24). Yet another group of cases, and perhaps the saddest of all, are the youngsters treated for nevus (11), keloid (11, 25), actinomycosis (17), or cystic hygroma (12), and to stop growth (26) of a limb of a boy stricken with polio. All of these developed osteogenic sarcoma 3–30 years after exposure to x-ray.

A very real probability exists that the successful utilization of radiation for the management of a cancerous condition may carry with it as a consequence of its therapeutic success the induction of a new neoplasm. There is a latent period between the exposure to irradiation and the development of cancer which may vary from 3–50 years. However, what is not known with any degree of certainty is the extent of the risk. Simply asked, it is this: The 50 cases of osteogenic sarcoma reviewed by the Memorial group (15), for example, represent a serious problem for the patients who developed the lesions, but how large was the population from which these cases were culled? Does this risk represent 10 percent, 1 percent, 0.1 percent or less (27)? When the evaluation of the risk for patients treated for a malignant condition is stated at even 0.1 percent, this small chance of late carcinogenesis is far outweighed by the probability of successful eradication of an otherwise fatal illness.

In the management of cancer all modalities of treatment carry a risk of morbidity and mortality. These are tolerable considering the disease entity. But what parameter of risk would be tolerable for the management of benign conditions, and particularly what parameter would be tolerable in the management of a benign condition which is not successfully treated by irradiation? With our present knowledge of ionizing radiation, is it acceptable to continue to irradiate bursitis with even a 0.001 percent risk of carcinogenesis?

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Amyloid Pseudotumor of the Lung

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Abstract

The thirty-ninth case of isolated nodular pulmonary amyloidosis and the twelfth case presenting as a solitary nodule is reported in this communication. The clinical and pathologic features of the disorder are reviewed, and suggestion is made for further immunologic studies.

Pulmonary involvement as the sole manifestation of primary amyloidosis is extremely rare. When found, it has involved either the tracheobronchial tree or the lung parenchyma. The latter form has occurred less frequently than the former.

The parenchymal form of involvement, in turn, may occur as multiple nodules or, as described in this report, a single nodule.

Case Report

L. T., a 55-year-old white female patient, was admitted to St. Vincent's Hospital after a fall which caused a fracture of the left femur. She was in apparent good health at the time of injury. Physical examination revealed signs suggesting a fracture of the left femur. Roentgenographic examination of the left leg confirmed the presence of a fracture of the left femoral neck. A companion roentgenographic examination of the chest showed plate-like atelectasis of both lung bases and a lobulated mass of 4 cm diameter in the left mid-lung field.

Review of a chest roentgenogram performed two years before did not show the mass. Laboratory studies including a complete blood count, urinalysis, urea nitrogen, blood sugar, electrolytes, alkaline phosphatase, serum calcium and phosphorous and protein electrophoresis were all negative or within normal limits. The sedimentation rate was elevated to 60 mm/hour.

Surgery was performed for reduction and immobilization of the femoral fracture.

The pulmonary lesion was now further evaluated. Pulmonary function studies were within normal limits. Bronchoscopy was normal and bronchial washings were P.C.I.

Thoracotomy was performed and a 4 x 5 cm mass was found in the superior and posterior basal segments of the left lower lobe. The left lower lobe was resected while the patient tolerated the procedure well.

Pathologic examination of the lower lobe of the left lung showed an ovoid, well-circumscribed, firm, greyish-yellow mass located in the superior and posterior basal segment of the left lower lobe. The mass was adjacent to but separated from the main stem bronchus and abutted the surrounding pleura.

Microscopic examination revealed the tumor to be composed of amyloid which was confirmed by appropriate histochemical staining reactions (Congo Red and Crystal Violet). The amyloid infiltrate completely replaced the involved parenchyma. The amyloid deposit was infiltrated by chronic inflammatory cells and multinucleated giant cells with ingested amyloid. At the edge of the amyloid there were aggregates of lymphocytes in follicular formation. No evidence of tuberculosis, fungi, or metastatic malignancy was found.

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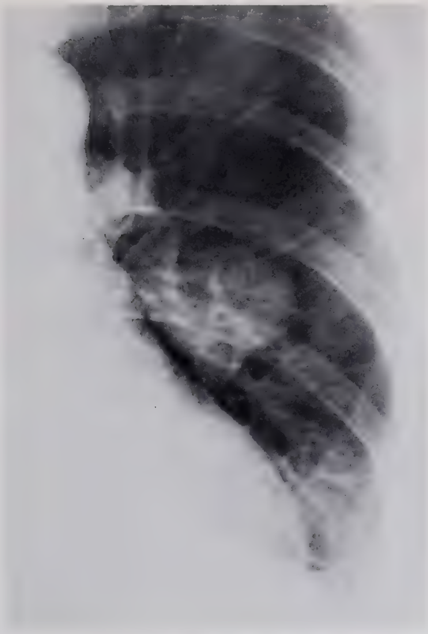

FIG. 1. Chest roentgenogram of left lung. There was a lobulated mass of 4 cm diameter in the left mid-lung field. Plate-like atelectasis was also noted.

Discussion

Amyloidosis is a degenerative disease which is caused by tissue deposition of amyloid fibrils. Recent evidence (1–4) has indicated that these fibrils are derived from light chain polypeptides of immunoglobulins. In general, amyloidosis is usually classified in the following manner (5):

1. Secondary amyloidosis—the most common form, which is associated with chronic infectious and/or chronic inflammatory disease.
2. Primary amyloidosis—a less common form without associated etiology. Primary amyloidosis may in turn be manifest as: a) a systemic form and b) a localized form.
3. Amyloidosis associated with myeloma.
4. Hereditary forms of amyloidosis.

All forms of amyloidosis may involve the respiratory tract. However, in primary amyloidosis respiratory tract involvement is common and usually

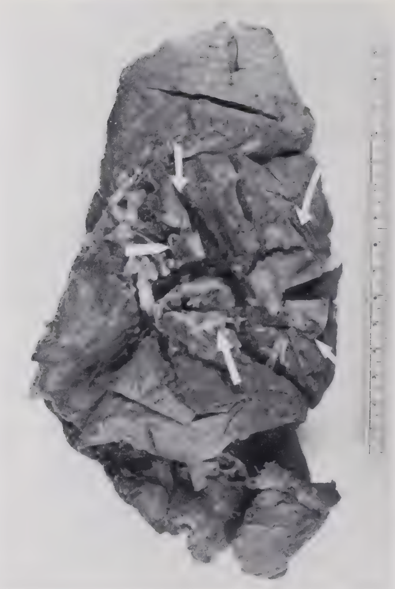

FIG. 2. Surgical pathologic specimen of lower lobe of the left lung. Pathologic examination of the lower lobe of the left lung showed an ovoid, well-circumscribed, firm, grey-yellow mass located in the superior and posterior basal segment of the left lower lobe. The mass was adjacent to but separated from the main stem bronchus and abutted the surrounding pleura.

causes clinical aberrations. A modification of the classification of Weiss (6) will help to clarify the types of respiratory tract involvement in primary amyloidosis:

A. Systemic Primary amyloidosis
   1. Nodular pulmonary involvement
   2. Diffuse pulmonary involvement
B. Localized Primary amyloidosis
   (Primary amyloidosis involving only the respiratory tract)
   1. Tracheobronchial amyloidosis
   2. Isolated nodular pulmonary amyloidosis

Systemic primary amyloidosis: In this form of amyloidosis the lung and heart are the organs that are most commonly involved. The lung is involved as part of the systemic process (7) in about fifty percent of cases. The deposits of amyloid fibrils occur in both the pulmonary and alveolar walls in either a

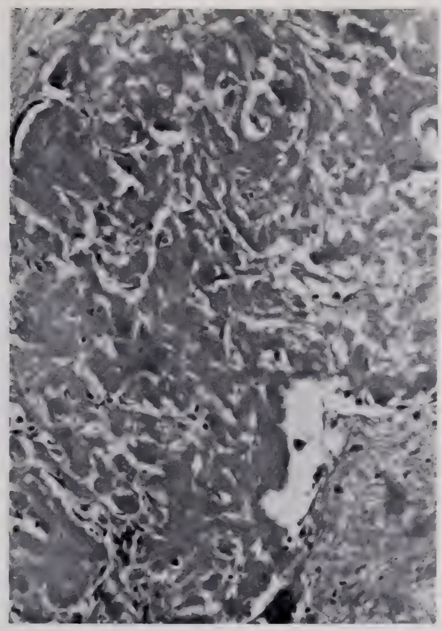

FIG. 3. Microscopic examination revealed the tumor to be composed of amyloid which was confirmed by appropriate histochemical staining reactions (Congo Red and Crystal Violet). The amyloid infiltrate completely replaced the involved parenchyma. The amyloid deposit was infiltrated by chronic inflammatory cells and multinucleated giant cells with ingested amyloid.

nodular or diffuse pattern. Marked pulmonary involvement may cause an alveolar capillary block syndrome.

Localized primary amyloidosis—Tracheobronchial: Amyloidosis may occur either with nodular or diffuse submucosal deposition of amyloid fibrils. Twenty-one cases (8) have been reported, including fourteen reviewed by Prowse (9) in 1958. The clinical manifestations of airway obstruction and bronchoscopic findings are distinctive.

Isolated nodular pulmonary amyloidosis may occur either as single or multiple deposits of amyloid. Thirty-eight case reports (10) have appeared in the medical literature as of February, 1974. Twenty-seven of these patients had multiple nodular involvement of the lung while eleven had solitary pulmonary nodules. The patient described in this report adds another case to the group with the solitary nodular form of the disease.

The clinical pattern of patients with solitary pulmonary amyloid nodules is rather typical (11, 12). They are usually older than fifty years of age (13). Most are asymptomatic: however, some have had cough (14) and a lesser number have had hemoptysis (15). No characteristic laboratory, bronchoscopic or pulmonary function abnormalities have been found. The solitary nodule can be found in any lobe of the lung and is usually small ($<$ 3 cm in diameter). The solitary pulmonary amyloid nodule shows no typical roentgenographic characteristics and must be considered in the differential diagnosis of "coin lesions" (16, 17). One patient reported by Dyke et al (18) had a recurrence of the amyloid nodule nine years after its original resection. Most other reported cases (19) have had no long-term follow-up so that the incidence of recurrence in the typical patient is unknown.

The diagnosis of isolated primary pulmonary amyloidosis depends on pathologic examination of the lesion. Grossly, the lesions are usually found within the lung parenchyma or beneath the pleura. They are discrete, firm, homogeneous, and translucent with a yellowish hue.

Microscopically the tumor usually consists of clustered aggregates of amyloid bordered by plasma cells, lymphocytes, histiocytes, and multinucleated foreign body giant cells. Calcification, cartilage formation, and ossification are sometimes found in the amyloid aggregates. Adjacent alveolar septa, blood vessels, and regional bronchioles may have amyloid deposition within their walls.

Isobe and Ossermann (20) have recently reported abnormal serum immunoglobulins in three cases of localized amyloidosis. In one, a monoclonal IgG was found in the sera and monoclonal IgM proteins were found in the sera of the other two. They do not mention the organ or tissue involvement in these patients nor do they mention if the lesions were solitary or multiple. They postulate that the source of the monoclonal immunoglobulins is the plasma cells adjacent to the amyloid deposits. It would be of interest to study the tissue of isolated amyloid lung nodules by immunofluorescent and immunochemical methods to pursue this postulate.

Therefore, we wish to suggest that radiologists, surgeons, and pathologists consider the possibility of this lesion when solitary nodules are found on chest roentgenograms. If the lesion is considered, then appropriate plans can be made for immunologic studies.

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Pulmonary Radiographic Findings in a Case of Febrile, Relapsing, Nonsuppurative Panniculitis (Weber-Christian Disease)

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Gilchrist and Ketron, Weber, and Christian reported cases characterized by subcutaneous nodular fat necrosis of the panniculus adiposus of unknown etiology in 1916, 1925, and 1928, respectively (1). Since then, approximately twelve autopsied cases of Weber-Christian Disease have been reported with skin and, additionally, systemic involvement (2–10). Only five of these had pulmonary involvement (2, 4, 7, 11, 12). One had only microscopically discernable pulmonary lesions. In four of the remaining case reports, descriptions of the chest x-rays were presented without illustrations. One was normal (11). In another the radiographic findings were not pertinent (2). Two were reported to show confluent, mottled, discrete, round infiltrations in the lower two-thirds of both lung fields (7). However, abnormal chest roentgenographs from a case of Weber-Christian Disease have, to the best of our knowledge, never appeared previously in the literature.

Clinically, the disease is characterized by tender, erythematous, subcutaneous nodules distributed over the extremities or the trunk, or over both. Fever and constitutional symptoms may be present, but their severity is related to the degree of systemic involvement. The illness is usually chronic with periods of remission and exacerbation. However, it may range from a brief benign disorder to a fulminating, terminal illness.

Case Report

First admission (6/12/63 through 6/29/63). S.M., a 45-year-old white man was admitted to University Hospital with the chief complaints of anorexia, nonproductive cough, easy fatigability since November 1962, and skin rash since May 1963. In March 1963, weight loss of twenty pounds, pharyngitis, and temperature rise to 101°F were noted. He had been treated prior to admission by his previous physician with intramuscular pencillin and diuretics for weight reduction. In April of 1963, fluffy lung infiltrations were noted in the left lower lung field. In May of 1963, many tender, nonerythematous, nonpigmented subcutaneous nodules were noted on the ventral and dorsal aspects of the extremities. Some of the skin lesions receded, the densities in the left lung receded, and his temperature returned to normal on the six-day course of steroid therapy administered immediately preceding his admission. There was no history of allergy, skin disease, pulmonary disease, or known occupational hazard. He was admitted to the hospital for diagnostic evaluation. Physical examination revealed an obese white male in no acute distress. The temperature was 99.8°F. The pulse rate was 72/minute and regular. The respirations were 16/min. The blood pressure was 130/90 mm Hg. A pharyngitis was present. Multiple, tender, firm, raised subcutaneous nodules measuring up to 1.2 cm in diameter were distributed over the neck and

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upper extremities, excluding the hands and thighs. The lymph nodes were not abnormally palpable. No hepatomegaly or splenomegaly was noted. The remainder of the physical examination was within normal limits. The positive laboratory tests were a mild leukopenia with some toxic granulation; serum protein cellulose gel electrophoresis showed alpha-l-globulin, 0.1 gm/100 ml (normal, 0.2-0.4 gm/100 ml); alpha-2-globulin, 0.2 gm/100 ml (normal 0.3-0.7 gm/100 ml); beta globulin, 0.8 gm/100 ml (normal, 0.6-1.1 gm/100 ml); gamma globulin, 1.5 gm/100 ml (normal, 0.7-1.4 gm/100 ml). The SMA-12 was normal, and the histoplasmin and Kveim skin tests were negative. An IVP showed a suspected mass in the left kidney, and a retrograde pyelogram revealed a large left kidney. The bone marrow was normal. A biopsy of the skin showed a "polymorphous infiltrate consisting of histiocytes, plasma cells and lymphocytes with occasional polys distributed chiefly around skin appendages suggesting a granulomatous pattern." Because of this pattern, the pathologist suggested the possibility of lymphoma. However, a definitive diagnosis was not possible at that time. Radiographs of the hands, wrists, long bones, and skull were within normal limits. A chest radiograph on 6/12/63 showed large coalescent, nodular, poorly circumscribed densities in both lower lung fields, and smaller densities in the upper lung fields. No pleural reaction was present. His clinical course as well as his chest radiograph were regressing to normal, and he was discharged to be followed at home.

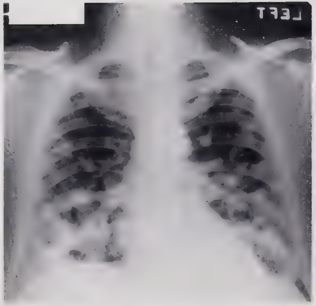
Second admission (11/10/63 through 12/25/63). The patient was readmitted because of temperature elevation to 102°F, night sweats, a productive cough, and a 25 lb. weight loss since his previous admission. He had not been taking any medication except Darvon®. His chest x-ray of 12/13/63 showed reappearance of the nodular densities (Fig. 1). He had a tender nodule on his right anterior chest wall. He also had 3+ ankle edema. A renal arteriogram was consistent with multiple infarcts. Lupus erythematosus preparations, histoplasmin, and blastomycin complement fixation tests were normal. The serum albumin was 2.8 gm/100 ml, and the serum globulin was 2.5 gm/100 ml. A biopsy of the skin nodule was consistent with nodular panniculitis of the Weber-Christian type. The white blood count was 3,200 cells/mm^3 with a normal differential. A bone marrow study was normal except for a slight increase in plasma cells. Therapy was initiated with 60 mg of prednisone, which was followed by a 13 lb diuresis, and marked but incomplete resolution of his pulmonary infiltrates. Administration of steroids was tapered to 5 mg per day, and the patient improved and was discharged.

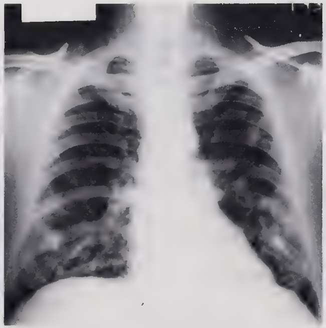
Third admission (12/29/64 through 3/17/65). He was admitted once again with disorientation, herpetic lesions of his lips, pain, a nodule at the base of his penis, and fever. The pulmonary nodules had recurred. Cerebral angiography and multiple spinal fluid analyses were compatible with a diffuse cerebritis. A sterile perineal abscess was incised and drained. A blood culture produced Listeria monocytogenes. Pencillin and tetracycline therapy was instituted and his steroid dosage was increased. As he improved his antibiotics were discontinued and administration of steroids was tapered. His chest radiograph showed remission with a few poorly defined nodular densities remaining. His discharge radiograph was similar to that of 1/20/64 (Fig. 2), which was during a stage of remission.

From the time of discharge to date, he has had mild exacerbations with recurrent changes in his chest radiograph, all responding to increased doses of prednisone. He has been followed periodically at our institution from discharge until 4/8/70, at which time a chest radiograph (Fig. 3) was essentially unchanged from 1/20/64.

Discussion

There have been so few autopsied cases of Weber-Christian Disease reported that it is difficult to find a unifying morphologic trend in the lesions that could clearly account for the previously reported pulmonary radiographic findings. It is furthermore perplexing to find pulmonary lesions associated with a disease which primarily produces fat necrosis in organs containing and anatomically intimately related to adipose tissue which is absent in the lung parenchyma (1). Nevertheless, we will try to summarize the previous autopsy





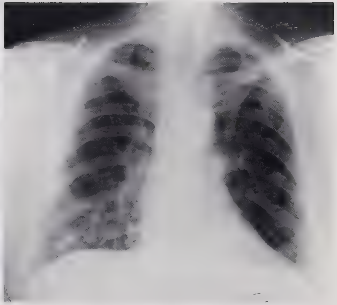


FIG. 1. PA Film of thorax on 12/13/63 shows numerous, fuzzy-bordered nodules in both lung fields of various sizes and mainly at bases.

FIG. 2. PA of chest on 1/20/64 shows marked resolution of infiltrates after cortisone, with some interstitial densities and some confluent densities mainly at bases.

FIG. 3. PA of chest 4/8/70 shows no essential changes from that of 1/20/64.

findings in the lung in order to give the reader some idea of what the morphologic correlation with the radiographic pattern might have been.

Two of the reported cases of pulmonary Weber-Christian Disease contained pulmonary fat emboli. In Milner and Mitchinson's case, numerous fat emboli were found most prominently near small, peripheral, necrotic lesions which were interpreted as pulmonary infarcts. However, no pulmonary thromboemboli were reported. The areas of necrobiosis contained free sundanophilic globules (4). A crescent-shaped layer of fat-filled macrophages and free fat were found in many alveoli distant from the area of necrosis. The authors suggested fat embolism as a possible explanation for the lung pathology. They proposed that the emboli might have originated in distant areas of adipose tissue destruction and carried a noxious substance to the lungs, resulting in inflammation and necrosis. The roentgenographic picture was similar to the roentgenograms illustrated in Figures 1, 2, and 3. The only pulmonary lesions found in the other case were a few fat emboli. The chest roentgenograph was reported to be normal (11). In contrast to the preceding cases, no fat emboli were found in the three remaining cases of Weber-Christian Disease with lung involvement. One showed 0.8-3 cm in diameter nodules of organizing lipogranulomatous pneumonitis in all lobes of both lungs (7). The roentgenographic picture described was also similar to the roentgenographs presented in this article. Within the lungs of the other case, vasculitis, perivascular and peribronchial granulomatosis, and organizing lipogranulomatous pneumonia were found (2). The chest roentgenographic findings were not pertinent. In another case report the chest roentgenographs were not described (12). Examination of the lungs at autopsy revealed some infiltration with lipophages. The presence of fat necrosis in the parietal pleura was not mentioned in any of the above cases. More cases of pulmonary involvement in this rare disease would have to be observed before any definitive statements could be made about the morphology or etiopathogenesis of the lung lesions.

The roentgen findings in our case consisted first of fluffy densities within the lungs, with the later development of nodules varying from several millimeters to several centimeters in diameter. The nodules with fuzzy margins were without segmental distribution but were primarily but not exclusively in the lower lung fields. Clinical regression with cortisone administration occurred as well as regression of the nodular densities which were replaced by mottled densities and at times by confluent mottled densities. The differential diagnosis is varied and multiple (13). Pulmonary radiographic findings associated with Weber-Christian Disease respond to cortisone therapy unlike bronchogenic spread of tuberculosis, blood-borne pyemic abscesses, fungus infection, lipid granulomata, metastatic carcinoma, sarcoma, multiple pulmonary hamartomata, and amyloid tumors. They also may be distinguished from metastatic carcinoma by the usual sharp borders seen at times in metastatic carcinoma. Lymphoma may present several features in common, but when multiple nodules in the lungs are present, mediastinal lymphadenopathy is apt to be quite striking. Wegener's granulomatosis, rheumatoid pulmonary nodules, lupus of the lungs and polyarteritis nodosa, Boeck's sarcoid, and the pulmonary infiltrations with eosinophilia syndromes are dependent on cortisone administration and have some roentgen similarity to the findings associated with Weber-Christian Disease. However, the tendency for cavitation of the lesions of the "vasculitis group" and the more striking involvement of the mediastinal lymph nodes in Boeck's sarcoid tend to help in the differentiation. Since pulmonary radiographic findings associated with Weber-Christian Disease are very rare initially, a differential diagnosis may be difficult, particularly from that of a lymphoma since the clinical picture and response to cortisone may be similar.

Although, we do not have a tissue diagnosis of the lung lesions in this case, we believe that the radiographic findings and clinical picture are so strikingly similar to those previously described (but never illustrated) that the case should be reported.

Summary

A case of systemic, febrile, relapsing, nonsuppurative panniculitis (Weber-Christian Disease) with associated chest roentgenographic findings is reported. The roentgen findings consisted first of fluffy pulmonary densities, primarily in the lower lung fields, with the later development of nodules with fuzzy borders. Clinical and roentgenographic regression occurred with cortisone administration. The nodular densities were replaced by separate, at times confluent, mottled densities.

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Metastatic Melanoma Presenting as Abnormal Uterine Bleeding: A Case Report

IRA S. JAFFREY, M.D., LOUIS LEFKOWITZ, M.D., STUART D. LESTCH, M.D., AND DANIEL R. WEISS, M.D.

Abstract

A case is presented in which a metastatic malignant melanoma presented as an enlargement of the uterus with abnormal uterine bleeding. The case is of particular interest because the patient manifested central nervous system metastases which distracted the physicians from the etiology of the uterine bleeding.

Uterine metastases from cutaneous melanoma are rare. Because of the unusual sequence of events in this individual, the following case is being reported.

Case Report

A 44-year-old caucasian female underwent resection of a malignant melanoma from the skin of the right upper back in February 1971. She remained asymptomatic until October 15, 1973 when she went to her gynecologist with abnormal uterine bleeding. At that time she was felt to have a uterus 6–8 weeks gestational size, and a tentative diagnosis of an aborting submucosal leiomyomata was made. The following day the patient presented herself at the hospital with a history of recent onset of headache associated with vomiting and lethargy. On neurologic evaluation she was found to have a right hemiparesis and right facial palsy. Physical examination revealed a 2 x $2\frac{1}{2}$ cm nodule inferior to the umbilicus in the right lower quadrant of the abdomen. X-ray studies revealed a right hilar mass measuring 2 cm in diameter. An electroencephalogram showed bilateral abnormalities more pronounced on the left side. The brain scan was within normal limits.

The abdominal wall nodule was discolored and showed a "halo" and was felt to be compatible with a metastatic malignant melanoma. An excisional biopsy was performed and the pathology report revealed an undifferentiated malignant tumor, compatible with metastatic malignant melanoma.

On day 1 the patient received 1.4 mg of Vincristine, 100 mg of BCNU and 400 mg of DITC by intravenous injection. On days 2 and 3, the patient received additional doses of BCNU and DITC also intravenously. There was a gradual improvement in the clinical state so that by day 8 she had regained function of the right upper and lower extremities and only partial evidence of the right facial palsy remained.

On day 9 the patient was taken to the operating room where a dilatation and curettage was performed. The pathology report of the cervical tissue revealed undifferentiated malignant tissue compatible with metastatic malignant melanoma.

On day 11 the patient was discharged from the hospital for follow-up as an outpatient for further chemotherapy.

On day 48 the patient was admitted to the hospital because of anemia and severe weakness of the left lower extremity which had been progressing over the preceding two weeks. At that time there was evidence of right facial palsy. In the hospital the patient was transfused with packed red blood cells and received a second course of combination chemotherapy. A myelogram was

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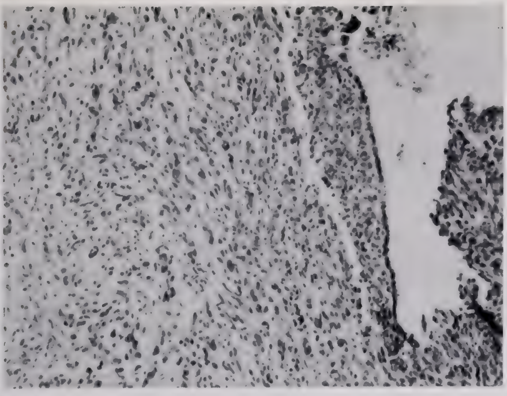

FIG. 1. Infiltration beneath endometrium.

performed which revealed a partial obstruction at the level of T_{11} and T_{12}. The patient was then transferred to another facility with the thought of administering Cobalt radiotherapy to this area. However the patient expired within 48 hours of admission, before therapy could be instituted.

At autopsy metastatic melanoma was found to involve the brain, spinal cord, right ovary, liver, pancreas, and myometrium.

Discussion

The review of the metastatic melanoma literature reveals few cases of uterine metastases from cutaneous melanoma presenting with vaginal bleeding. In this case the diagnosis was delayed because of the onset of symptomatic central nervous system metastases. It is unusual for the uterus to be the site of primary or metastatic malignant melanoma, although the latter is more common than the former.

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Student Section

Lateral Chest Wall Cyst in a Woman with Disseminated Echinococcal Disease

LEON S. HARRIS, A.B.

A case is reported of a 74-year-old Israeli-born woman with disseminated echinococcal disease who had a slowly enlarging cystic lesion of the left lateral chest wall. The 9cm x 7cm cystic mass covered the middle and posterior axillary lines, was tender, and surrounded by local soft tissue swelling; extension to the pleural cavity could not be excluded.

The epidemiology, clinical symptomatology, diagnosis, and treatment of echinococcal disease are reviewed. The variety of presenting signs and symptoms and the necessity for careful removal of hydatid cysts are discussed.

Case Report

B.B., a 74-year-old Israeli-born white woman was admitted to The Mount Sinai Hospital for removal and drainage of an echinococcal cyst on the left lateral chest wall as part of further treatment of disseminated hydatid disease.

The initial operative procedure 15 years prior to admission was a right nephrectomy, which was followed by recurrence of cysts in the liver, spleen, and left kidney. Ten years prior to admission the excision of a hepatic cyst was accompanied by spillage and intraperitoneal dissemination. Subsequently, the patient developed intrathoracic cysts with hemoptysis and expectoration of cyst material, left axillary vein thrombosis due to cyst pressure, and the passage of cyst material in the urine. One year prior to admission the patient spontaneously ruptured an epigastric hepatic cyst through the skin, which then healed.

Several months prior to admission the patient noted a slowly enlarging cystic mass over the left lateral chest wall. Recently the patient had noted respiratory difficulty with chronic cough, a single episode of bloody sputum one week before entry, shortness of breath, and a slight left chest wall pain. There was no history of weight loss, diarrhea, or passage of cysts in the stool.

The patient was admitted for removal and drainage of a large recurrent echinococcal cyst of the left lateral chest wall with possible extension to the pleural cavity.

On physical examination the patient was afebrile. Decreased breath sounds were noted at the left base posteriorly, with bronchophony and egophony, and occasional expiratory wheezing at the left interscapular region. A 9cm x 7cm left lateral chest wall cystic mass was noted, covering middle and posterior axillary lines, slightly tender without signs of inflammatory changes on the skin. Also noted were multiple small cystic masses near the areas of old abdominal scars. Chest x-ray confirmed the presence of the cyst, with surrounding soft tissue swelling.

The urine contained 5-6 RBCs/hpf and many WBCs. The hematocrit was 37.6, the white blood cell count 11,300. The sodium was 138 mEq/L, chloride 97 mEq/L, potassium 5.2 mEq/L, CO_2 29 mEq/L, BUN 27 mg%, and glucose 106 mg%. The alkaline phosphatase was 104 King-Armstrong

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units, total bilirubin 0.3 mg%, cholesterol 237 mg%, SGPT 12 units/ml, SGOT 17 units/ml, CPK 81 sigma units/ml, total protein 8.5 gm%, and albumin 4.1 gm%.

The patient refused consent for external drainage of the cyst and was discharged on the seventh day after admission.

Discussion

Discussion of echinococcal disease conveniently can be divided into the following areas: the life cycle of the tapeworm, epidemiology, pathology of the lesions, clinical symptomatology, diagnosis, and treatment.

Life Cycle. Echinococcal or hydatid disease is caused by the tapeworms Echinococcus granulosus or Echinococcus multilocularis (Fig. 1). The adult worm lives in the small intestine of dogs, wolves, coyotes, and foxes, and is acquired by eating the organs of animals which contain the cyst stage with its numerous scolices.

In the canine or definitive host, except for extremely heavy infections, the worm usually causes no harm. Eggs released from the terminal proglottid of the worm are passed in the feces of the infected dog, and subsequently are eaten by the intermediate host, which is usually sheep but can also be cattle, horses, or man. The embryo passes through the intestinal wall of these intermediate hosts, and then via lymphatics or mesenteric venules travels to various body parts where within six months cysts (approximately 10 mm in diameter) may develop. Thus man, though he may be involved in the cyst

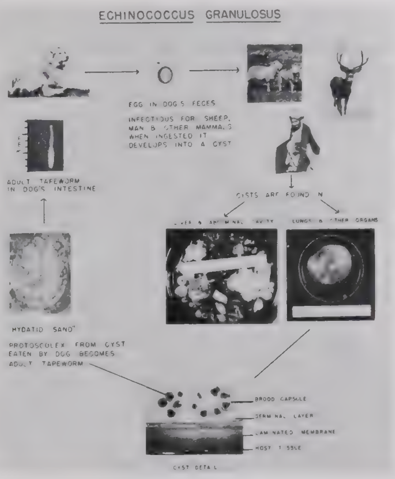
ECHINOCOCCUS GRANULOSUS
EGG IN DOG'S FECES
INFECTIOUS FOR SHEEP, MAN & OTHER MAMMALS WHEN INGESTED IT DEVELOPS INTO A CYST
ADULT TAPEWORM IN DOG'S INTESTINE
CYSTS ARE FOUND IN
LIVER & ABDOMINAL CAVITY
LUNGS & OTHER ORGANS
HYDATID SAND"
PROTOSCOLEX FROM CYST EATEN BY DOG BECOMES ADULT TAPEWORM
BROOD CAPSULE
GERMINAL LAYER
LAMINATED MEMBRANE
HOST TISSUE
CYST DETAIL

FIG. 1. Life cycle of Echinococcus granulosus (with permission from Brown: *Basic Clinical Parasitology*, 4th Ed., 1975, Ref. 3).

stage of the disease, does not complete the full cycle because his infected organs are not eaten by dogs.

The most common type hydatid cyst in man is unilocular and grows slowly over several years. If uninfluenced by local pressure effects they are usually spherical, approximately 1–7 cm in diameter, but with a range up to 20 cm.

The morphology of the cyst consists of (Fig. 2):

1. an external, laminated nonnucleated supporting layer;
2. an inner, nucleated, germinal layer;
3. colorless or light yellow sterile fluid;
4. brood capsules, with only the germinal layer and containing protoscolices; and
5. daughter cysts which are replicas of the mother cysts. If a daughter cyst ruptures, the protoscolices released into the hydatid fluid become known as "hydatid sand."

The protoscolices are important for two reasons. First, the numerous protoscolices (up to two million) contained in a single fertile cyst can evolve in the gastrointestinal tract into numerous adult tapeworms. Secondly, if the cyst ruptures within the host, the protoscolices may become widely disseminated and develop numerous daughter cysts.

In summary, the definitive host (usually dog) consumes the viscera of infected larval hosts and thus becomes infected; tapeworms develop and release their eggs in the intestinal tract of these definitive hosts. The intermediate or larval host (sheep, man) swallows the eggs passed in the feces of the definitive host; the embryo forms, develops, and passes through the intestinal mucosa, and infection with hydatid results.

Epidemiology. Since dogs are the usual definitive hosts, and sheep and cattle the primary intermediates, human disease is highest in sheep and cattle-raising countries that use the help of dogs in herding. Those areas most

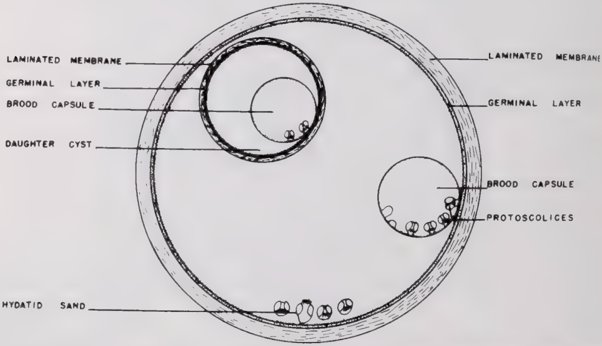

FIG. 2. Hydatid cyst of Echinococcus granulosus, diagrammatic (with permission from Brown: *Basic Clinical Parasitology*, 4th Ed., 1975, Ref. 3).

affected are Africa, the Middle East, South America, Australia, and New Zealand.

In North America hydatid disease is most prevalent in Alaska, where up to 22% of dogs are infected. Wolves, moose and caribou serve as the intermediate hosts, and close contact between dogs and humans is common. A focus has also been reported in California among Basque sheepherders. In general, the distribution and incidence in any area parallels the development of the livestock industry.

Disease in Alaska differs from the classic type, and distinction between the two is important for the diagnosis, management, and treatment of hydatid disease. In the European, or classic form, the disease is most prevalent in livestock-raising countries where domestic animals, dogs, sheep, or cows are the hosts. Infection is usually clinically symptomatic (80–95% of the time), and complications are frequent, including cysts outside the liver and lung, metastatic seeding, giant cysts, anaphylaxis, and fistula formation. Because of the prevalence of symptomatic infection and the frequency and severity of possible complications, the surgical approach to this form of the disease must of necessity be an aggressive one.

In the form prevalent in Alaska and Canada, the hosts are wild animals, wolves, moose, or caribou. Disease in these northern regions rarely has complications and, in fact, is usually asymptomatic, with symptoms present in only 6–8% of cases. Therefore, the basic approach to this variant of the disease state often can be a conservative one. Thus, in North America, it is important to determine which form is present in any individual case since the more benign variant in Alaska and Canada, being less symptomatic, with fewer complications, allows a more conservative, often nonoperative approach. Differentiation is aided by a history of residence in an area where either type of host population is abundant.

Pathology and Clinical Symptoms. Clinical symptomatology is entirely dependent on the location of the cysts, the major sites being the liver 66%, lung 22%, and kidney, brain and bone each about 2%. At each site where an embryo lodges, an inflammatory reaction of mononuclear cells and eosinophils with encapsulating fibrous adventitia develops. Some embryos from the portal drainage are initially destroyed in the liver, while those that are not may become cysts. The symptoms are determined by the location of the enlarging cysts, with the resulting local tissue destruction, atrophy, and pressure necrosis of neighboring tissue cells. Thus, the site, type and rate of cyst development is important in terms of the damage done. Most strains grow very slowly, and though infection often takes place in childhood the clinical manifestations are not usually apparent until years after the original infection, often middle age, and the presentation will be of a primary hepatic, pulmonary, central nervous system, or osseous lesion.

Considering various sites of involvement, abdominal cysts cause increasing abdominal discomfort, but not until the cysts have reached a large size. One of the characteristic features of hydatid disease is often the extent of organ involvement and the long existence of the cyst before symptoms develop.

Hepatic cysts are usually primary, three-fourths in the right lobe often along the inferior surface growing downward into the abdominal cavity. Many are asymptomatic for years until finally presenting as palpable abdominal masses, abdominal pain, or obstructive jaundice from pressure on the biliary tree.

Ruptured cysts can release free protoscolices with spread of daughter cysts either by direct extension or by bloodstream. Causes of rupture include coughing, muscle strain, blows, aspiration, or operative procedures. Rupture may be into bile ducts, peritoneal cavity, lung, pleura, or bronchi, and may lead either to an anaphylactic reaction which may be fatal if the rupture is massive enough, or to secondary dissemination of echinococcal infection.

With pulmonary involvement rupture of peribronchial cysts can discharge cyst material into a bronchus. Usually the rupture is incomplete and can go on to form a chronic pulmonary abscess which is susceptible to secondary infection with pyogenic bacteria. Symptoms consist of the sudden onset of coughing accompanied often by allergic symptoms, and the production of sputum containing frothy blood, mucus, hydatid fluid, and bits of membrane. Other early symptoms may be slight hemoptysis, dyspnea, transient chest pain and pruritus.

Central nervous system involvement may be revealed either with symptoms of increased intracranial pressure and symptoms caused by local pressure effects on vital centers, or with epilepsy perhaps caused by local irritative effects.

Renal cysts may give flank plain, hematuria, and kidney dysfunction, while rupture of a cyst may result in hydatid fluid and cyst material being passed in the urine.

With osseous lesions there is histologically a foreign body giant cell reaction, with destruction of trabeculae, osseous necrosis, and spontaneous pathologic fractures. The earliest lesions are often in the epiphysis and spread is via destruction of trabeculae and growth along planes of least resistance.

Diagnosis. Diagnosis is based on the presence of a slowly growing cystic (especially hepatic) tumor, history of residence in an endemic area, and close association with dogs or other hosts. Echinococcal disease is rarely thought of first, and thus the differential diagnosis includes malignancies, abscess, cirrhosis, or any primary lesion of whatever organ is involved. The clinical presentation is often not clear enough to suggest the diagnosis, but several helpful indications are listed:

a) eosinophilia, present in 20–25% of cases;
b) x-rays showing pulmonary cysts or calcified cysts in any location, particularly hepatic;
c) liver scan showing a space-occupying cystic lesion;
d) the Casoni skin test; and
e) serologic tests, either the indirect hemagglutination test or the bentonite flocculation test.

The Casoni intradermal skin test is useful for diagnostic and epidemiologic purposes, though at present there is need for better standardization of antigen dosage and test technique. The antigen is sterile hydatid fluid in a dose of 0.5 or 1.0 ml, though there is no adequate qualitative standard dose, and often a specific antigen type is needed for the diagnosis of disease originating from a specific area. There are both immediate and delayed reactions, the immediate reaction being a better indicator to monitor. An immediate negative is good evidence of freedom from disease, but a positive reaction persists for years even following the removal or death and calcification of a cyst. The test is 86% positive, with 18% false positives often a result of other worm infections. Thus, while a positive reaction denotes disease in the present or past, the Casoni skin test is most useful when a negative immediate reaction rules out the existence of hydatid disease.

The serologic tests of choice are presently the indirect hemagglutination test and the bentonite flocculation test. There is a complement fixation test which, though less sensitive than the other two tests, reverts back to normal rapidly with the removal of the cysts. Thus its main use can be in postoperative evaluations, with a positive complement fixation 6 to 12 months postoperatively often meaning the presence of a second cyst. Both the indirect hemagglutination and bentonite flocculation tests are 90% positive with hepatic involvement, though only 40% positive with lung involvement. A definitive diagnosis can be made if high enough titres are present, greater than 1:400 in the hemagglutination and greater than 1:5 in the flocculation test.

The major drawback is an incidence of 25% false positive due to cirrhosis, lupus, nephrosis, and other clinical situations, but these can usually be dismissed on clinical grounds. Thus the indirect hemagglutination test, with conservative interpretation and an awareness of its limitations, can provide a definitive diagnosis.

Treatment. Treatment is surgical. There are no successful medical or chemotherapeutic modalities available. Because the outer laminated membrane of the cyst is very fragile, conventional methods of cyst removal can result in rupture. This should be strenuously avoided because of two important dangers; that hydatid fluid in contact with host tissues can lead to an anaphylactic reaction, and secondly that the liberated larvae can cause widespread secondary disease throughout the host. One method is to expose, carefully wall off with black towels, aspirate, sterilize, and then excise the cyst. Saidi and Nazarian describe another method by which a metal cone with a circulating coolant flowing through it is placed on the surface of the cyst to create a rigid, frozen ring. They then evacuate through an isolated central portion and sterilize with 0.5% Ag nitrate (a formalin solution is used by others to sterilize).

It should be remembered that the symptoms which were present were due to pressure effects of the enlarging cyst. If only a single cyst was present, and if it was adequately removed without leakage, the host organ can often fully recover its function.

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Assessing Patient Knowledge about Diabetes

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Abstract

This paper reports the results of a multiple-choice questionnaire administered to 99 patients in the Diabetes Clinic at City Hospital Center at Elmhurst, New York during a 5-week period in May and June, 1975. The questionnaire was designed to assess levels of information possessed by clinic users in the following areas: use of insulin, urine testing, hygienic self-care, diabetic diet, and symptoms of insulin shock and ketoacidosis. Social, demographic and other personal characteristics were also determined for correlation with performance on the questionnaire.

Mean and median scores on the questionnaire both were 47%. Patients scoring lower tended to be older, less well educated, in treatment at the clinic for less than three or more than nine years, Spanish-speaking, and reliant exclusively on the clinic for information about their disease. Personal experience with insulin use and with acute complications of diabetes was associated with better scores on questions in these areas.

The general lack of patient information about diabetes revealed in this study raises important questions concerning patient compliance, quality of care, and cost-effectiveness of treatment for this clinic population. Recommendations are offered to improve the clinic's ability to fulfill its educational function.

Introduction

Patient health education is, to borrow from the title of a recent journal article, "an idea whose time has come" (1). Whereas life expectancy increased by 17 years, from 54 to 71, during the 40-year period ending in 1960, there was little if any improvement during the 1960s. Despite vast increases in expenditures for health care and continuing growth of useful medical knowledge, available indices of illness, disability, and premature death actually worsened for some groups during this period. The "missing link" is now felt by some experts to be adequate levels of patient understanding and compliance with physicians' instructions (2, 3).

Improved health education is particularly important in the prolonged management of chronic diseases. In acute-care problems, patients may take a relatively passive role in the therapeutic process. In chronic diseases such as diabetes, the patient's role is more likely to fit the "mutual participation" model of Szasz and Hollander (4). Doctor and diabetic patient should work together, with the patient assuming responsibility not only for taking medications as prescribed, but also for managing important daily activities —eating, exercising, choosing what type of shoes to wear—according to a specific plan designed in cooperation with the doctor. Diabetics should be able to adjust their therapy according to predetermined guidelines, such as changing insulin dosage to compensate for changes in eating or exercise habits

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on weekends (5). Patients should also understand how to utilize available health services to their own best advantage.

Diabetics frequently do not attain a satisfactory level of joint participation with their physicians (6). Acutely ill patients assume a "sick role" that is generally limited in duration and provides its own inducement for compliance. Well-controlled diabetics, on the other hand, may not be sick, and must consciously assume an "at-risk" role which lasts a lifetime (7). Compliance with a carefully planned management program is regarded by many diabetologists as a critical factor in the long-range course of diabetic patients:

> In many patients, the late complications of diabetes may be lessened and/or postponed by good dietary regulation and the early and proper use of insulin. It is the large population of so-called "mild," often obese, diabetics without dietary regulation or adequate insulin treatment that provides the great majority of diabetics who, in later years, develop coronary artery disease, gangrene, retinopathy, and/or nephropathy (8).

One might expect to find low levels of compliance among patients with a relatively asymptomatic chronic disease. A recent review of published accounts of diabetic compliance concluded that "Overall, less than 10% of the patients observed were carrying out a minimally adequate regimen." (9) The health care system thus would seem to have failed to meet the needs of 90% of diabetic patients.

Poor compliance among diabetic patients may reflect poor understanding about diabetes and the rationale of treatment. Motivation requires more than knowledge, to be sure, and a well-informed patient may not always follow a doctor's advice. However, patients who do not understand the substance and implications of medical advice will be unlikely to follow it (10).

Several studies of patient compliance have demonstrated a correlation with levels of understanding. Both compliance and understanding are directly associated with a patient's level of formal education, often cited as the most useful determinant in predicting health information and health status (11, 12). Other studies have demonstrated an inverse relationship between patients' understanding and their age at the time of assessment (13).

The first aim of this study was to assess the level of information about diabetes possessed by patients receiving continuing care in the Diabetes Clinic at City Hospital Center at Elmhurst (CHCE).

A second aim was to determine whether any sociodemographic or other personal characteristics were associated with low levels of understanding of diabetes.

The setting for the study was the Diabetes Clinic at CHCE, a Mount Sinai School of Medicine municipal hospital affiliate which serves some 850,000 residents of the Borough of Queens, New York. With an estimated national prevalence of 14.5 per thousand in the general population, diabetes can be assumed to afflict approximately 12,300 persons in the Elmhurst service area. Approximately 800 patients are currently enrolled in the Elmhurst Diabetic Clinic. All have had at least one 2-hour postprandial serum glucose level in excess of 140 mg% (8, 14).

Materials and Methods

In consultation with internists, nurses and dieticians working in the Diabetes Clinics both at Elmhurst in Queens and at The Mount Siani Hospital in Manhattan, a questionnaire was developed to test patients' knowledge of diabetes. This questionnaire included 30 multiple-choice questions covering five areas in which we considered patient understanding to be important in successful management: general knowledge about diabetes, diabetic diet, practical aspects of ongoing treatment programs (including urine testing and hygienic care), the use of insulin, and recognition of insulin shock and diabetic coma. Wording of questions was as nontechnical as possible. Synonyms for medical terms were used freely. Copies of the questionnaire were made available both in English and Spanish.*

Questions were pretested with a group of five randomly selected clinic patients, and in several cases items were eliminated because few patients responded correctly. The final questionnaire was reviewed by two attending physicians in the Elmhurst Diabetes Clinic. The questions were judged to be reasonable, and it was felt that most diabetic patients should be able to answer them (14).

Following these 30 questions on diabetes was a series of items requesting personal and demographic data. This information included: age, formal education, number of years with known diabetes, number of years under treatment at the CHCE Diabetes Clinic, previous treatment in the CHCE General Medical Clinic or by a private doctor, primary language, and ethnic group. Finally, patients were asked about their current therapy, past experience with insulin shock or diabetic coma, and whether any family members or close friends with diabetes had been a source of information to them.

The questionnaire was administered at seven consecutive Monday morning and Friday afternoon clinic sessions from May 16 through June 9, 1975. All 133 patients who attended the Diabetes Clinic during that period and who had been in treatment at the clinic for at least two years were asked to complete the questionnaire. Of 133 patients, 99 completed three or more of the questions on diabetes.

Motivating patients to complete a written questionnaire was difficult. Several steps were taken to deal with this problem. The individual distributing the questionnaires personally introduced himself to each patient. The purpose of the project was briefly described as an evaluation of patient education about diabetes. Patients were told that their names were not required, and that their doctor would not see their completed questionnaires. Directions for completing the questionnaire were carefully explained, and assistance was given in interpreting the meaning of specific questions or words. In some cases, part of the questionnaire was read aloud to patients in order to encourage completion. Patients were allowed help from their spouses, on the assumption that the spouse might be responsible for management of diet and therapy in the home.

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* A copy of the questionnaire is included in the Appendix. Percentages of correct answers for each item are included for the 99 patients participating in this study. The assistance of Mrs. Violet Hayes and Mr. Angel Morales of the Mount Sinai Medical Center in preparing the Spanish version is gratefully acknowledged.

The questionnaire was distributed in the clinic waiting area, before patients saw their doctor. In many cases the questionnaire appeared to provide a welcome diversion. Several patients spontaneously approached the nurses to ask for a copy. A final inducement for completing the questionnaire was the promise that a copy of the correct answers would be supplied to patients, and any questions explained, after the questionnaire had been completed and returned.

Results

Of 133 patients asked to participate, 34 either refused or completed less than three items. Reasons for refusing varied. Some patients stated that they could not see well enough to read or that they could not speak English or Spanish, while others were simply not interested. Because there was no time limit, unanswered questions were scored as incorrect. The final study group included 99 patients.

For the 30 questions on diabetes, the mean number of correct answers was 14.2 (47%). The median score was 14 of 30 (47%). Scores were divided into three roughly equal groups for correlation with demographic variables (Table I).

Previous studies (13) have noted an inverse relationship between age and understanding of health problems. This study population ranged in age from 18 to 79 years, with a "young" group aged 18 to 50, a "mid" group aged 51 to 64, and an "old" group aged 65 to 79. The median age of 58.5 is consistent with Reilly's finding of a median age range of 50 to 64 in another sample of 86 patients drawn from the same clinic (15).

Table II indicates an inverse relationship between age and performance on the questionnaire. Of the young group, more than three times as many scored in the top third (13) as in the bottom third (4). Performance of the old group was evenly divided among top, middle and bottom thirds, while scoring of the mid-age group fell between that of the young and the old. The 16 patients who did not give their ages scored predominantly in the bottom third.

Other studies (11, 12) have demonstrated that higher levels of formal education are associated with better understanding of health problems. The patients in this study were divided into three groups according to formal

TABLE I

Breakdown of Scoring

Group	No. of Patients	No. of Correct Answers	Score
Top Third (TT)	32	18–25	60–83%
Middle Third (MT)	35	12–17	40–57%
Bottom Third (BT)	32	3–11	10–37%

education: 0–8th grade (26), some high school (21), and high school graduates (30). Included in the last group were two patients who had attended college, two college graduates, and one who had attended graduate school.

Table III shows the relationship between education and performance on the questionnaire. Among high school graduates, nearly four times as many fell in the top third (15) as in the bottom third (4). For the 0–8th grade and high school groups, performance was fairly evenly divided between top, middle and bottom thirds.

Table IV shows the relationship between age and educational level. Older patients included a high percentage with little formal education. This suggests that poor questionnaire performance of older patients may reflect a generally lower level of education.

The median duration of treatment in the clinic was four years. These data are comparable to those obtained by Reilly (15). The relationship between

TABLE II

Age vs Performance

Performance	No. (%) of Patients by Age Group			
	Old	Mid	Young	Totals
BT	9 (31)	4 (15)	4 (15)	17
MT	9 (31)	15 (56)	10 (37)	34
TT	11 (38)	8 (30)	13 (48)	32
Totals*	29	27	27	83

* 16 respondents did not report their age.

TABLE III

Education vs Performance

Performance	No. (%) of Patients by Educational Group			
	0–8th Grade	Some HS	HS Grad.	Totals
BT	7 (27)	5 (24)	4 (13)	16
MT	11 (42)	8 (38)	11 (37)	30
TT	8 (31)	8 (38)	15 (50)	31
Totals*	26	21	30	77

* 22 respondents did not report their education.

TABLE IV

Age vs Education

Education	No. (%) of Patients by Age Group			
	Old	Mid	Young	Totals
0–8 Gr.	12 (55)	5 (23)	2 (12)	19
Some HS	3 (14)	6 (27)	8 (47)	17
HS Grad.	7 (32)	11 (50)	7 (41)	25
Totals*	22	22	17	61

* 38 respondents failed to supply age and/or education.

years enrolled in the clinic and performance on the questionnaire is shown in Table V. Comparison of the scores of 32 patients more recently enrolled in clinic (2-4 years) with those of the 36 patients enrolled for 5 or more years shows that recent enrollees scored nearly as frequently in the bottom third as in the top (10 versus 12). On the other hand, those enrolled for five or more years scored in the top third twice as often as in the bottom third (12 versus 6).

The median time since the diagnosis of diabetes was nine years. Since patients may have learned about diabetes from personal experience before enrollment in this clinic, the relationship between questionnaire performance and time since diagnosis was examined. Table VI indicates that fewer patients in the two- to six-year group scored in the top third than in the bottom third (7 versus 10) with a distinct reversal of this ratio in patients followed for longer periods.

Many patients followed in the Diabetes Clinic are concurrently being followed in the CHCE General Medical Clinic (GMC). Of 81 respondents, 58 (72%) stated that they had attended the General Medical Clinic at some time. Reilly found that 42% of Diabetic Clinic patients had attended GMC within the previous twelve months (15). Table VII shows the relation between GMC attendance and questionnaire performance. Of GMC attenders, the TT/BT ratio was 2.3, while for GMC nonattenders this ratio was 0.74.

Of 81 respondents, 24 (30%) stated that health professionals other than those at the Diabetes Clinic had helped them to understand diabetes. All but three of these health professionals were identified as physicians. The data presented in Table VIII do not suggest a striking correlation between

TABLE V

Years at Diabetes Clinic vs Performance

Performance	No. (%) of Patients by Years at Clinic						
	2	3–4	Total 2–4	5–9	10+	Total 5+	Totals
BT	5 (39)	5 (26)	10 (31)	3 (16)	3 (18)	6 (17)	16
MT	4 (31)	6 (32)	10 (31)	9 (48)	9 (53)	18 (50)	28
TT	4 (31)	8 (42)	12 (38)	7 (37)	5 (29)	12 (33)	24
Totals*	13	19	32	19	17	36	68

* 31 respondents did not indicate years at the clinic.

TABLE VI

Years with Diabetes vs Performance

Performance	No. (%) of Patients by Years with Diabetes			
	2–6	7–12	15+	Totals
BT	10 (37)	5 (19)	3 (14)	18
MT	10 (37)	9 (35)	9 (43)	28
TT	7 (26)	12 (46)	9 (43)	28
Totals*	27	26	21	74

* 25 respondents did not indicate years with known diabetes.

TABLE VII

Attendance at General Medical Clinic vs Performance

Performance	Attended GMC (%)	Did Not Attend (%)	Totals
BT	11 (19)	8 (35)	19
MT	22 (38)	9 (39)	31
TT	25 (43)	6 (26)	31
Totals*	58	23	81

* 18 respondents did not indicate GMC attendance.

TABLE VIII

Consultation with Other Health Professionals vs Performance

Performance	No. (%) of Patients Seeking Other Professional Consultations		
	Other Consultations	None	Totals
BT	3 (13)	15 (26)	18
MT	10 (42)	22 (39)	32
TT	11 (46)	20 (35)	31
Totals*	24	57	81

* 18 respondents did not indicate consultations with other health professionals.

TABLE IX

Fluency in English vs Performance

Performance	No. (%) of Patients Fluent in English		
	Fluent	Not Fluent	Totals
BT	15 (23)	6 (33)	21
MT	23 (35)	9 (50)	32
TT	27 (42)	3 (17)	30
Totals*	65	18	83

* 16 respondents did not indicate fluency in English.

consultations with health professionals outside the Diabetes Clinic and questionnaire performance.

As indicated in Table IX, 18 of 83 respondents (22%) indicated that they were not fluent in English. All 18 identified Spanish as their primary language. However, since the questionnaire was distributed only in English and Spanish, patients unable to read either language were necessarily excluded from this study. Among the 34 patients who did not participate, seven declined because of a language barrier. This group included patients whose primary languages were Greek, Russian, French, Chinese, and Serbo-Croatian.

Although the size of the non-English-speaking sample is small, there appears to be a strong relationship between fluency in English and questionnaire performance. The top third in performance included 42% of the fluent-in-English group, with the TT/BT ratio being nearly 2 to 1 for those fluent in English, as compared to 1 to 2 for those not fluent in English.

Patients may be more apt to answer correctly those questions which have special pertinence to their own experience with diabetes. For example, diabetic patients now using insulin may be expected to have a better understanding of the use of insulin and of the symptoms and causes of diabetic coma and insulin shock, as compared to insulin nonusers. Table X indicates the performance of insulin users versus nonusers of 9 insulin-related questions (questions 1, 14, 24-30), and shows that patients using insulin performed better on this group of questions than did patients not using this therapy. With the exception of question 25, which is pertinent to all diabetic patients, all the above questions were answered correctly more frequently by insulin users.

However, comparison of the performance of insulin users to nonusers is not the only use of these data. Four of 31 insulin users sampled were not aware that injection sites should be rotated, and 25 of the 31 were unaware that alcohol intake can present special risks. Low performance scores on these questions indicate specific areas where better patient education is needed. Moreover, it is interesting to note in Table XI that insulin users did not have appreciably higher overall performance scores.

TABLE X

Insulin Treatment vs Performance on Insulin-Related Questions

No. (%) of Patients Answering Correctly			Specific Knowledge Tested
Question Number	On Insulin	Other Treatment	
1	25 (81)	30 (63)	Insulin lowers blood sugar
14	27 (87)	28 (59)	Injection sites should be rotated
24	2 (71)	22 (46)	Diabetic coma and insulin shock require MD management
25	10 (32)	17 (37)	Overeating and stress can cause diabetic coma
26	14 (45)	3 (7)	Vomiting, diarrhea, undereating, overexercise can cause insulin shock
27	12 (39)	9 (19)	Symptoms of insulin shock
28	15 (48)	15 (31)	Symptoms of diabetic coma
29	15 (48)	10 (21)	Insulin shock and diabetic coma are specific risks to diabetic patients
30	6 (19)	6 (12)	Alcohol use is a special risk to insulin-dependent diabetics
Totals*	31	47	

* 21 patients did not specify current therapy.

TABLE XI

Insulin Treatment vs Performance

Performance	No. (%) of Patients Now Using Insulin		
	Yes	No	Totals
BT	8 (26)	6 (15)	14
MT	7 (23)	21 (54)	28
TT	16 (52)	12 (31)	28
Totals*	31	39	70

* 29 respondents did not complete this item.

TABLE XII

Experience with Shock or Coma vs Performance on Questions Relating to Acute Complications

No. (%) of Patients Answering Correctly			Specific Knowledge Tested
Question Number	Coma or Shock	No Coma or Shock	
24	16 (80)	27 (47)	Diabetic coma and insulin shock require MD management
25	8 (40)	20 (34)	Overeating and stress can cause diabetic coma
26	7 (35)	9 (15)	Vomiting, diarrhea, undereating, overexercise can cause insulin shock
27	8 (40)	13 (22)	Symptoms of insulin shock
28	9 (45)	20 (34)	Symptoms of diabetic coma
29	8 (40)	16 (27)	Insulin shock and diabetic coma are specific risks to diabetic patients
30	3 (15)	8 (14)	Alcohol use is a special risk to insulin-dependent diabetics
Totals*	20	58	

* 21 patients did not specify experience with diabetic coma or insulin shock.

It is reasonable to expect that patients who have personally experienced diabetic coma or insulin shock will do better on questions dealing with these acute complications of diabetes. Questionnaire performance data on this issue are presented in Table XII. These data indicate a clear relationship between questionnaire performance and past experience with insulin shock and diabetic coma. Again, it must be emphasized that four of 20 patients who had experienced these serious complications failed to understand that they are emergency conditions requiring physician management. Further, it is notable that no other questions in this group were answered correctly by even 50% of patients who had experienced insulin shock or diabetic coma.

Patients who had a close friend or family member with diabetes did not perform better on the questionnaire, nor was there any clear relationship between questionnaire performance and specific therapy.

Discussion

This questionnaire has demonstrated the potential utility of an objective technique for assessment of patient's understanding of diabetes. Both average and mean scores on the questionnaire were only 47%. Thus, there appear to be serious deficiencies in the understanding of diabetes, its natural history, and its management among this group of patients in the Elmhurst Diabetes Clinic. Patients scoring lower tended to be older, less well educated, in treatment at the Clinic for less than three or more than nine years, less frequent users of other clinics or private physicians, and less likely to be fluent in English.

Several factors may contribute to an association of younger age with better performance on the questionnaire. Diabetics over age 65 are at greater risk of reduced cerebral function and may begin to lose either intellectual powers or interest in self-care. In addition, juvenile diabetics might be expected to have more severe disease and would thus be more apt to seek out health information. There may also be the effects of a cohort phenomenon, in that those in the young age group tend to have had more formal education and, presumably, more recent exposure to formal schooling and multiple-choice examinations. Finally, it is possible that physicians and nurses may devote more effort to educating younger diabetic patients.

Similar factors may be partly responsible for the higher scoring of patients with more advanced formal education, as indicated by the high degree of correlation between age and education. The relationship between performance and years of treatment indicates that patients may, in fact, be increasing their understanding as a function of time spent in clinic until, at approximately nine years, increasing age tends to counterbalance the educational benefit of long-term enrollment.

Access to alternate sources of medical care may not have been directly responsible for the better performance of patients utilizing them, despite the associations demonstrated. Patients who attend another clinic or see a private doctor may be more highly motivated and, like juvenile diabetics, may be more apt to make special efforts to learn about their disease. In order to investigate this issue further, it would be useful to administer the same questionnaire to diabetic patients attending General Medical Clinic who may not be concurrently using the Diabetic Clinic. No attempt was made to obtain this additional information during the present study.

The poorer scoring of patients unable to communicate freely in English may in fact be a direct result of the language barrier. Of the three regular clinic physicians (two attending physicians and one resident), only one spoke even broken Spanish.

In view of the association, outlined in the introduction to this paper, among ignorance, noncompliance, and diabetic complications, the data indicate that this population—especially the demographic groups scoring poorly—is at serious risk. Diabetes is currently a focus of organized patient education efforts in many community hospitals (2). The need for increased efforts in this area at Elmhurst is clear from the results of this study.

An instrument similar to this questionnaire could be used more extensively for determination of knowledge about diabetes or, in modified form, for assessment of patients' knowledge about other chronic diseases. As indicated by the results presented, educational needs may vary both with the patient and with the type and stage of his disease.

The data indicate that currently the major role in patient education is assumed by clinic physicians. The contribution of other health professionals would appear not to be recognized by patients. The role of the nurse in the management of diabetic patients can be expanded to include the distribution of literature and identification cards, provided free by pharmaceutical companies and the American Diabetes Association, to every new patient enrolled. On several occasions during the conduct of this study, new patients in the Diabetes Clinic failed to receive any such written information, although informational literature is available both in English and Spanish. Nurses and nurse-practitioners are also in a good position to provide, with physician supervision, both initial orientation and ongoing instruction. More ambitious educational programs may involve additional outlay of funds. A program at the University of Southern California has demonstrated a high level of cost-effectiveness, and might serve as a model for hospitals elsewhere (16).

Another mode of patient education is through patient-to-patient teaching. The American Diabetes Association has endorsed the concept of a "contract" wherein patients, in return for the treatment they receive, pledge to do their part in the education process (17). The best educational potential may be realized when such programs are managed by professional health educators. The American Association of Diabetes Educators has recently advocated using such professional educators to supervise the orientation of new patients by other diabetics on a one-to-one basis (5).

The greatest obstacle to the establishment of sound educational programs has been the failure of third-party payers to provide reimbursement for such services. However, change may be imminent. The President of the Blue Cross Association has recently advocated greatly expanded patient education services, and a bill has been introduced in Congress to provide significant public funding for consumer health education (3). Strong support for this bill has been received from the American Hospital Association and the American Association of Medical Colleges (1). Pilot programs in effective techniques of patient education may provide an important contribution to the development of a more substantive national policy of consumer health education in the future.

Appendix*

This questionnaire is part of an effort to evaluate how well *we* at the Diabetes Clinic are doing to help you understand diabetes. It is *not* an examination of you, and the doctors and nurses will not know how well you score. It is important for you to do your best, however, in order for us to evaluate ourselves accurately.

Directions: FOR EACH QUESTION PLEASE CIRCLE THE NUMBER NEXT TO THE *ONE* BEST ANSWER TO THE RIGHT OF THE PAGE.

	EXAMPLE: In diabetes the body is unable to use (water/minerals/sugar) properly.	water 1 minerals 2 sugar③
68 (21)	1. Insulin (raises/lowers/has no effect on) the level of sugar in your blood.	raises 1 lowers② has no effect on 3
79 (5)	2. Without diet or treatment, a diabetic's blood sugar would be (too high/too low/normal).	too high① too low 2 normal 3
57 (16)	3. In general, diabetes which begins in childhood is (more severe/less severe/about the same severity) compared to adult-onset diabetes.	more severe① less severe 2 about the same severity 3
28 (15)	4. In the United States there is an average of one diabetic in every (1,000/50/5) persons.	1000 1 50② 5 3
44 (9)	5. In general, diabetics should (inform their family of their diabetes/inform close friends and co-workers of their diabetes/carry a card with specific information/do all of these/do none of these).	inform their family 1 inform close friends and co-workers 2 carry a card 3 do all of these④ do none of these 5
66 (8)	6. The goal of treatment in diabetes is to (cure the disease/control the complications/produce no sugar in the blood).	cure the disease 1 control the complications② produce no sugar in the blood 3

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* Correct answers are circled. Percentage of respondents answering each item correctly is underlined in left-hand margin. Percentage of respondents failing to answer each item is in parentheses.

47 (5)	7. All diabetics should (take insulin or pills/take insulin and pills/watch their diet/all of these/none of these).	take insulin or pills 1 take insulin and pills 2 watch their diet③ all of these 4 none of these 5
33 (18)	8. With proper treatment, diabetics probably (will nevertheless have some more health problems than other people/should plan not to have a family/will die of diabetes/all of the above/will have no more health problems than other people).	some more health problems than others① should plan not to have a family 2 will die of diabetes 3 all of the above 4 no more health problems than others 5
75 (7)	9. Urine tests should be done (by all diabetics/only when treatment has been recently changed/only by doctors and nurses).	by all diabetics① only when treatment recently changed 2 only by doctors and nurses 3
70 (9)	10. An increase in the amount of sugar in your urine usually means the sugar in your blood has (gone up/gone down/remained the same).	gone up① gone down 2 remained the same 3
20 (21)	11. In order to test your urine with Clinitest tablets, you must (place ten drops of urine in the bottle of tablets/swallow one tablet/add one tablet to ten drops of urine/add one tablet to dilute urine/dissolve one tablet and inject the solution).	place 10 drops urine in bottle of tablets 1 swallow one tablet 2 add one tablet to 10 drops urine 3 add one tablet to dilute urine ④ dissolve tablet and inject solution 5
42 (17)	12. If you do more exericse, you are likely to (increase/decrease/cause no change in) the need for dietary control or drugs.	increase 1 decrease② cause no change in 3
52 (24)	13. If you have an infection you are likely to (increase/decrease/cause no change in) the need for dietary control or drugs.	increase 1 decrease 2 cause no change in 3
65 (24)	14. When injecting insulin, a diabetic should (inject in the same place each time/inject in a different place each time/inject in the same place each time and eat something immediately after the shot).	same place 1 different place② same place and eat something 3
35 (17)	15. As a diabetic you are more likely than others to have (trouble with your vision, infections or abscesses, and kidney disease/trouble with your vision and infections or abscesses/trouble with your vision and your kidneys/trouble with infections or abscesses only/trouble with your vision only).	vision, infections or abscesses, and kidney ① vision and infections or abscesses 2 vision and kidney 3 infections or abscesses only 4 vision only 5
43 (13)	16. If you begin to get a blister on your foot after wearing a new pair of shoes, you should (put iodine on the blister and stop wearing the shoes/put iodine on the blister and tell your doctor/stop wearing the shoes and tell your doctor/put iodine on the blister, stop wearing the shoes and tell your doctor/stop worrying about it and allow the blister to heal normally).	iodine and stop wearing shoes 1 iodine and tell your doctor 2 stop wearing shoes and tell your doctor③ iodine, stop wearing shoes and tell your doctor 4 stop worrying and allow to heal normally 5
34 (20)	17. A diabetic person should *never* eat which one of the following foods? (rice/vanilla ice cream/sweet potatoes/bread/marmalade).	rice 1 vanilla ice cream 2 sweet potatoes 3 bread 4 marmalade⑤
26 (15)	18. As a diabetic, you can eat as much as you want of which of the following foods? (bananas/cooked mushrooms/onions/clear broth/dietetic jelly).	bananas 1 cooked mushrooms 2 onions 3 clear broth④ dietetic jelly 5
80 (7)	19. As a diabetic, you should always (have at least one hot meal per day/have at least one cold meal per day/have at least 3 meals per day).	at least one hot meal 1 at least one cold meal 2 at least 3 meals③

66 (13)	20. As a diabetic, you can have snacks (never/only if you eat less for other meals/as much as you want).	never 1 only if you eat less for other meals② as much as you want 3
39 (14)	21. If you suddenly feel very ill and dizzy, but do not know whether your sugar is too high or too low, you should (go to bed/take insulin or pills/drink orange juice with sugar/take insulin or pills and call a doctor/drink orange juice with sugar and call a doctor).	go to bed 1 take insulin or pills 2 drink orange juice with sugar 3 take insulin or pills and call a doctor 4 drink orange juice with sugar and call a doctor⑤
29 (18)	22. Which of the following combinations are in the same dietary exchange group? (corn and beets/lima beans and bread/meat and potatoes).	corn and beets 1 lima beans and bread② meat and potatoes 3
45 (11)	23. If your diet allows you one teaspoon of margarine, you can (use one teaspoon of margarine in cooking/use one teaspoon of margarine on bread/both of the above/either one or the other but not both/none of the above).	one teaspoon in cooking 1 one teaspoon on bread 2 both of the above 3 either one or the other, but not both④ none of the above 5

IN QUESTIONS 24-30, CIRCLE THE NUMBER 1 IF THE STATEMENT APPLIES ONLY TO *DIABETIC COMA* (HIGH BLOOD SUGAR, KETOACIDOSIS). CIRCLE THE NUMBER 2 IF THE STATEMENT APPLIES ONLY TO *"INSULIN" SHOCK* (LOW BLOOD SUGAR, INSULIN REACTION). IF THE STATEMENT APPLIES TO *BOTH*, CIRCLE THE NUMBER 3.

53 (22)	24. Serious condition(s) requiring prompt attention of a doctor.	diabetic coma 1 "insulin" shock 2 both③
35 (35)	25. Caused by over-eating, infection, surgical operations or other stress.	diabetic coma ① "insulin" shock 2 both 3
19 (36)	26. Caused by vomiting, diarrhea, too little food or unusual exercise.	diabetic coma 1 "insulin" shock② both 3
25 (37)	27. Onset sudden; person feels excited, hungry, and has pale, moist skin.	diabetic coma 1 "insulin" shock② both 3
36 (35)	28. Person breathes hard, feels thirsty and sick to his stomach, and has high level of sugar in his urine.	diabetic coma① "insulin" shock 2 both 3
29 (34)	29. Happen(s) to diabetics more than to other persons.	diabetic coma 1 "insulin" shock 2 both③
14 (37)	30. Especially likely to happen if a diabetic who is taking insulin drinks alcoholic beverages.	diabetic coma 1 "insulin" shock② both 3

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Tribute to Dr. Louis J. Soffer on the Occasion of the Dedication of the "Louis J. Soffer Endocrinology Laboratory"*

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In 1949, in the midst of my internship, I was forced to suspend my activities to convalesce from an attack of hepatitis. On my way to my sickbed I stopped at a bookstore to purchase half a dozen contemporary classics in medicine. One of these was Dr. Louis J. Soffer's book entitled *Diseases of the Adrenals.* I had never heard of Dr. Soffer before, but as I read this book, I felt "like some watcher of the skies when a new planet swims into his ken." This was the book that ignited my own lifelong interest in the adrenal cortex. Since that time I have referred to Dr. Soffer's book repeatedly and have always found it to be authoritative, complete, accurate, and lucid. For us grant-addicted endocrinologists of the 1970's, it should serve as a reminder that scholarship in endocrinology was possible in the 1940's, before there were any federal subsidies for endocrinologic research.

Although he has been unaware of it, this early introduction to Dr. Soffer's work has given me a lively interest in everything he has written since that time. I have admired him not only for his research contributions but also for what he has represented in American medicine. Louis Soffer's name for many years has been synonymous with integrity, with wisdom, and with sensitivity to academic and human values. How many young people have benefited from his kindly encouragement and his example of excellence! How many of us here today have found in him an inspiring role model! I consider it one of the greatest honors of my life to have been asked to participate in the dedication of the laboratory that will stand as a lasting monument to what Dr. Soffer has meant to academic endocrinology in general and to Mount Sinai School of Medicine in particular.

Dr. Soffer's career has spanned a period of spectacular progress in biomedical science, a period in which powerful new diagnostic and therapeutic tools have been placed in the hands of the medical profession. His career has exemplified the fusion of science and humanism that constitutes the dual heritage of "the doctor." Years ago, one aspect of this heritage found perfect expression in Sir Luke Fildes' famous old painting of "The Doctor" that depicted the compassionate physician keeping vigil over a critically ill child. A sense of helplessness and despair pervaded the sickroom of that simple home. The painting conveyed the impression that the physician is our best, last hope in times of illness; yet, even the most skillful and compassionate physician is limited in what he can do.

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* The "Louis J. Soffer Endocrinology Laboratory" was dedicated at Mount Sinai School of Medicine of The City University of New York, on June 18, 1975.

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For me, Sir Luke Fildes' doctor has always had personal meaning. In 1935 my younger sister was stricken with an illness characterized by chills, fever, cough with pink frothy sputum, shortness of breath, and pleurisy. Our family physician came to see her, and after completing his examination he told us that she had "lobar pneumonia." She should be treated with mustard plasters; otherwise there was little we could do but await the so-called "crisis" in eight more days. To my untutored mind, this meant that on the eighth day she would either die or recover. Those were eight anxious days, made worse by the knowledge that in those days "pneumonia" was the most common cause of death in America. How the picture has changed since then! Now pneumococcal pneumonia has been reduced to a minor medical problem as a consequence of Alexander Fleming's marvelous discovery of penicillin.

Dr. Soffer's career is testimony to the fact that the doctor is not alone in his compassionate concern for suffering humanity. Compassion is the animus, the motivating force that vitalizes all of medicine. Sublimated compassion is the animus, the motivating force that vitalizes biomedical research. The objective of biomedical research is to provide clearer insights and better tools to enable practicing physicians to do more for their patients. It is only through research that physicians acquire the tools and insights that are necessary to solve the problems of their patients. To illustrate this fact, I have taken notes from the records of ten patients recently discharged from Vanderbilt University Hospital (Table I). Each suffered from an incapacitating illness, many potentially fatal. All of them left the hospital either cured or substantially improved. In the Table, I have listed on the left the major diagnoses of these ten patients. On the right I have listed the major discovery or technologic development that made possible the physician's successful intervention in each case. Without the discoveries of basic scientists the physicians would have been powerless to alter favorably the courses of their patients' illnesses.

TABLE I

Major diagnosis	Basic discovery
1. Disseminated histoplasmosis	Amphotericin B
2. Meningococcemia	Penicillin
3. Uremia	Immunosuppression and transplantation
4. Aortic insufficiency	Prosthetic valves
5. Gout	Allopurinol
6. Cholecystitis	Asepsis and anesthesia
7. Hemophilia	Cryoprecipitate
8. Pernicious anemia	Vitamin B_{12}
9. Diabetic coma	Insulin
10. Drug overdose	Respirator

Several of these basic discoveries were made by scientists who had no inkling that their discoveries would ever be applied to the diseases in question. The scientists who have made such contributions are legion. Most of them were searching for "truth," an understanding of nature, without any thought that their discoveries would someday be applied by others to the problems of clinical medicine. Since the clinician can rarely foretell whence might come the solution to his patient's problems, it is in the best interest of medicine to have as rich a body of science to draw upon as possible. Communications must be kept open between clinicians and scientists of all disciplines so that whatever facts and techniques *are* available can be applied without delay to the solution of patients' problems.

We are sometimes challenged to prove the "relevance" of basic science to the solution of clinical problems. During the past decade, anti-intellectual forces from both the right and the left have argued that they see no relevance of "basic research" to medical practice. But science is always potentially relevant to clinical medicine if it is engaged in a search for understanding of human biology. Without the advancement of basic science and its application to clinical practice, our physicians of the future cannot expect to be better than those of the present.

Those of you who are members of the medical honorary fraternity Alpha Omega Alpha know that its motto is, "Worthy to serve the suffering." To be worthy to serve the suffering implies more than a willingness to serve, for a willingness to serve the suffering is shared alike by the educated physician, the social worker, the witch doctor, and the faith healer. What sets the educated physician apart from the others and makes him worthy to serve the suffering is his superior understanding of human biology, for only through such understanding comes the power to make a specific diagnosis, to offer an accurate prognosis, and to recommend specific therapy.

Although we have indeed made much progress, every physician still all too often finds himself faced with insoluble problems, in a world "where men sit and hear each other groan, where palsy shakes a few sad last gray hairs, where youth grows pale and specter-thin and dies, where but to think is to be full of sorrow and leaden-eyed despairs." Every physician still faces problems that lie beyond his comprehension, beyond his power to solve. He still needs help, and who is there to help him? There are voices in the land that say, "We don't need more knowledge . . . we need doctors . . . not necessarily *good* doctors . . . *any* kind of doctors. Let us stop supporting research and training, and have all of our doctors take care of patients." Forty years ago they would have said, "Out, out, Alexander Fleming! Stop playing with your fungi and start applying mustard plasters; there are people all around you in the great city of London who are in pain from pleurisy and empyema." *I say we need our compassionate practitioners, but we also need our basic scientists, and we need laboratories in which they can work.*

In dedicating the Louis J. Soffer Endocrinology Laboratory, we honor a man who has always seen clearly the interdependence of clinical medicine and biomedical science. We dedicate this laboratory to science, firm in our conviction that biomedical science represents sublimated compassion and that it is through the wise and humanistic application of science that we gain power to alleviate suffering, to enable others to live and to live more abundantly. Surely any less lofty ideal would be unworthy of the name of Louis J. Soffer.

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Asbestos Exposure of Brake Repair Workers in the United States*†

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Introduction

At least 900,000 people in the United States are employed as auto mechanics or garage workers (1). An unknown but large percentage of these workers regularly service brake linings. Brake linings pose a potential hazard for asbestos exposure because they contain 33–73% asbestos (2–4). Jacko and Ducharme (2) have estimated that 70 million pounds of asbestos (32 million Kg) are worn away from brake linings each year in the United States. Much of the asbestos worn away (around 80%–90%) drops to the road or is emitted into the atmosphere.

The wearing of asbestos linings is complex and for brief periods of time local areas in the lining surface have temperatures exceeding 630°C, the temperature at which chrysotile asbestos loses the major portion of its water of hydroxylation and loses its characteristic morphology. The bulk temperature cannot exceed 350–400°C for any length of time without decomposition of the organic polymer binder material.

Several investigators (2–4) have analyzed bulk brake-drum dust for chrysotile and have found weight percentages of between 0.3% (2) to "at most 1%" (3). Lynch (4) reported percentages of 10% and 15% free fiber in two of fifteen samples. Most of the rest were below 1%.

Personal and background sampling of workers engaged in brake maintenance work at the job site gives a more direct estimate of exposure.

During brake-lining servicing the wheel is removed and all loose dust is removed from the drums and backplates. Compressed air jets are usually used (Fig. 1). In a two-city survey in the USA, Castleman et al (5) found that 175 out of 220 establishments used this procedure. Alternates include vacuuming and wet brushing. The brake lining itself may require grinding to remove irregularities or removal and replacement. The new lining may require considerable manipulation to fit the brake shoe—beveling edges and punching holes in the material, for example. As an alternative, the brake shoe and lining

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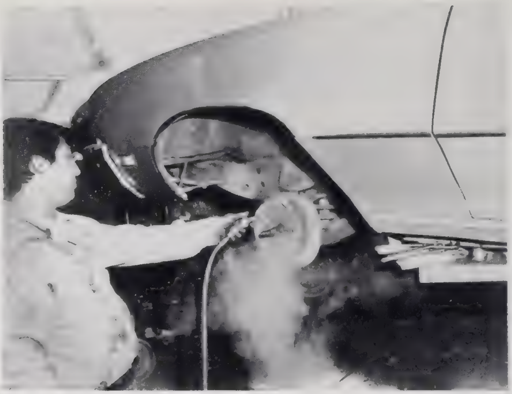

FIG. 1. Auto worker cleaning brake drum with compressed air jet. Note personal sampler on the right side of worker's chest.

may be replaced as a unit. A number of studies have measured exposure in such work, and fiber counts reported are summarized in Table I.

In addition, Boillat and Lob (8) reported values for various manipulations of the brake-lining material including punching holes for rivets, and grinding. Sampling interval is not given. Fiber counts ranged from 0.3 to 29.2 fibers/ml; four of the nine values were over 5 fibers/ml (5,000,000/m^3).

Asbestos disease: Asbestosis in brake liners has been recognized and compensated in Great Britain for many years (9), and instances of mesothelioma in garage workers have also been identified (10–12). Boillat and Lob (8) examined 39 vehicular maintenance workers who had performed brake-lining service for about ½–2 hours a day for a mean of 8± years. They found two men who may have had mild cases of asbestosis. The study. however, provided no information as to the risk with longer exposures.

Present Investigation

In order to provide additional information on this subject, we have investigated asbestos exposure among brake repair maintenance workers in New York City and have initiated a clinical survey of workers employed in the workshops studied.

ASBESTOS EXPOSURE

Materials and methods

Personal air samples were taken during brake-lining maintenance work both on automobiles and trucks. These were peak samples taken over 2–10 minutes

TABLE I

Asbestos Fiber Measurements in Brake Maintenance Service

Reference	Operation	Sampling method	Sampling interval (min)	Fibers/ml^3	Peak or TWA*	Comments
Hickish and Knight (3)	blowing out dust	static samples by side of car-2	90	1.25	TWA*	Four brake blowouts during this time
		static samples, in dust cloud during blowing-2	90	2.55		
	mechanic employed on brake cleaning, blowing	personal sampling (2 men)	450	0.68	TWA	—Eight hour shift
			100	7.09		—Work on brakes
			300	0.08		—Not working on brakes
	background	pairs of static samples (morning and afternoon)	180	0.28	TWA	Brakes blown in morning but not in afternoon
	—adjacent bay		180	0.07		
	—2 bays away		180	0.17		
			180	0.07		
	—garage center		180	0.49		
			180	0.11		
Hatch (6)	blowing out dust	peak samples over a few secs. 7 samples from dust clouds	few seconds	43 ± 2.1	Peak	Hand-held pump
Knight and Hickish (7)	blowing dust	personal (two men)	60	5.35		Taken over hour during which brake work done
				0.84		
		from breathing zones (2 men)	2 secs.	87	Peak	pump in breathing zone
				87		
		static sampler adjacent bay	60	0.52		
				0.16		

* TWA = Time weighted average

during which the workers were performing certain tasks, such as blowing dust from drum brakes, renewing used linings by grinding, and beveling new linings. Background samples were also taken at varying distances and times. The standard techniques for filter processing and fiber counting which have been adopted by the Occupational Safety and Health Administration of the US Department of Labor were used (13). Samples of dust collected in the standard manner were examined both by standard optical techniques (fibers/ml $> 5\mu$) and by electron microscopy to give $\mu g/m^3$ of air, and the results compared. Bulk samples of brake-drum dust were collected and analyzed by electron microscopy. One hundred fibers were sized in each sample. Qualitative morphologic comparisons of the fibers with standard chrysotile morphology were made.

Results

Fiber concentrations for personal and background samples during blowing dust from drum brakes on automobiles are presented in Table II. The values show extensive variation, but the values at 3–5 feet are by far the highest, with a mean of 15.9 fibers/ml.

Fiber concentrations for personal and background samples during renewing used linings by grinding truck brakes are presented in Table III. The mean concentration for the personal sampler was 3.8 fibers/ml. Fiber concentrations

TABLE II

Personal Samples—Automobile Brake Service

Operation	Distance	Concentration (fibers/ml)*
Blowing dust out of drum brakes	3–5 ft.	13.8
Blowing dust out of drum brakes	3–5 ft.	29.4
Blowing dust out of drum brakes	3–5 ft.	6.6
Blowing dust out of drum brakes	3–5 ft.	13.6
Blowing dust out of drum brakes	5–10	3.8
Blowing dust out of drum brakes	5–10	2.0
Blowing dust out of drum brakes	5–10	4.2
Blowing dust out of drum brakes	10–20 ft.	0.4
Blowing dust out of drum brakes	10–20 ft.	4.8

Background Samples—Automobile Brake Service

	Distance from Operation	Time Lapse	Concentration (fibers/ml)
Blowing dust out of drum brakes	10 ft.	0 min.	0.3
Blowing dust out of drum brakes	20 ft.	0 min.	0.8
Blowing dust out of drum brakes	12 ft.	5 min.	0.2
Blowing dust out of drum brakes	50 ft.	5 min.	0.1
Blowing dust out of drum brakes	65 ft.	7 min.	0.1
Blowing dust out of drum brakes	75 ft.	14 min.	0.1

* Only fibers longer than 5 μ were counted by phase microscopy at 400×.

TABLE III

Personal Samples—Truck Brake Service

Operation	Concentration (fibers/ml)
Renewing used linings by grinding	2.7
Renewing used linings by grinding	7.0
Renewing used linings by grinding	2.2
Renewing used linings by grinding	4.1
Renewing used linings by grinding	5.0
Renewing used linings by grinding	4.7
Renewing used linings by grinding	5.6
Renewing used linings by grinding	1.7
Renewing used linings by grinding	2.5
Renewing used linings by grinding	2.0

Background Samples—Truck Brake Service

Operation	Distance	Concentration (fibers/ml)
Background to grinding used linings	10 ft.	1.2
Background to grinding used linings	10 ft.	1.7
Background to grinding used linings	25 ft.	1.0
Background to grinding used linings	25 ft.	0.6
Background to grinding used linings	60 ft.	0.2

for personal and background samples during beveling new linings for trucks are given in Table IV. The mean concentrations were 37.5 fibers/ml.

To determine the correlation between optical fiber counts and mass of chrysotile, sections of filters collected as above were analyzed both by phase contrast optical microscopy and transmission electron microscopy. The results are given in Table V. Ten samples of bulk brake-drum dust were analyzed to determine the percentage of short fibrils, defined as 250–500 Å × 750–3750 Å. A mean of 83% of all chrysotile fibers were in this category. A mean of about 20% of the total mass of the ten samples was chrysotile by electron diffraction. Throughout the examination by electron microscopy, attention was given to the morphology of the fibers. A majority of fibers showed little alteration in the typical chrysotile fiber (see Figure 2). Some fibrous and granular particles, when analyzed by electron microscope-electron microprobe techniques, had structural and chemical characteristics of fosterite, a thermal alteration product of chrysotile (Fig. 3). Amorphous material with magnesium silicate composition was also found along with the fibrous particles (Fig. 4).

CLINICAL STUDY

Materials and methods

104 men, members of a union of vehicular maintenance workers, were examined in a preliminary study of brake-maintenance workers in New York City. All were volunteers. The examination included a detailed occupational and environmental history, medical history, physical examination, chest

TABLE IV

Personal Samples—Truck Brake Service

Operation	Concentration (fibers/ml)
Beveling new linings	31.7
Beveling new linings	23.7
Beveling new linings	32.7
Beveling new linings	72.0
Beveling new linings	26.3

Background Samples—Truck Brake Service

Operation	Distance	Concentration (fibers/ml)
Background to beveling linings	8 ft.	0.6
Background to beveling linings	12 ft.	0.5
Background to beveling linings	12 ft.	0.3
Background to beveling linings	30 ft.	0.3

TABLE V

Automobile Brake-Lining Service

Operation	Comparison of Optical and E. M. Fiber Counts	
	Optical Microscopy	Electron Microscopy
	(fibers/ml)	*μg/m³*
Light grinding of new linings before installation	4.8	53.0
Grinding new linings before installation	2.7	66.0
Blowing dust off drum with air jet	0.4	1.1
Cleaning brake drum with hand brush	3.6	6.5
Background to blowing out brake drum (10 feet away)	0.3	0.2
Background to blowing out brake drum (20 feet away)	0.8	0.1
Background to blowing out brake drum (65 feet away)	.1	0.2

x-ray, and pulmonary studies. Three men were excluded from analysis because they had worked in a shop for less than 10 years. Also excluded from analysis were nine men whose occupational history raised the possibility of other asbestos exposure. Two men were excluded because of known chest disease. The remaining 90 believed themselves to be healthy; all either were currently working (84) or had retired because of age (6).

Occupational Categories

General mechanics	67
Body workers	7
Undercoaters	5
Lubrication	4
Painters	2
Miscellaneous: other crafts	5
	90

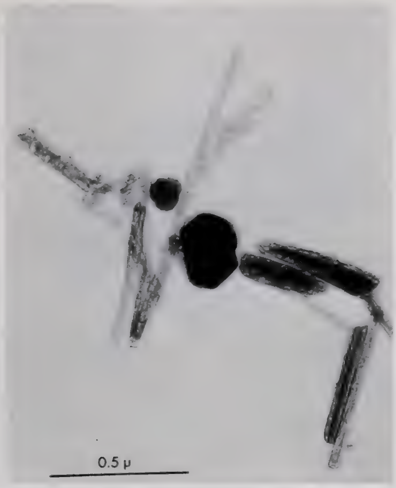

FIG. 2. Chrysotile fibers from brake-drum dust.

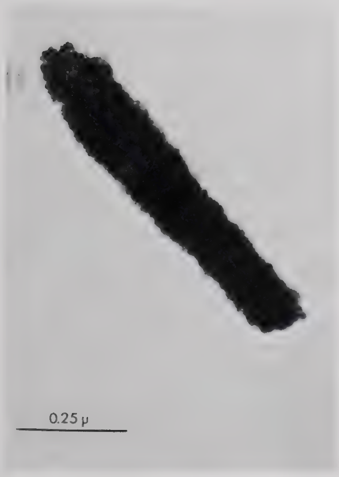

FIG. 3. A fosterite fiber from brake-drum dust.

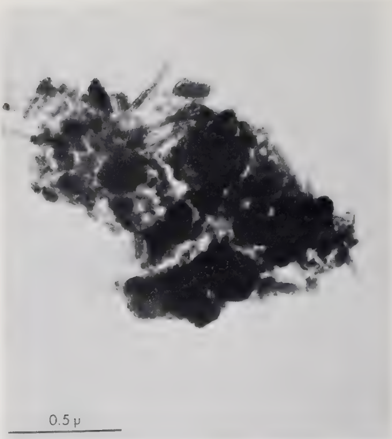

FIG. 4. Amorphous material mixed with chrysotile fibers from brake-drum dust.

Sixty-one of the sixty-seven general mechanics did brake lining more than once a week for ten years or more. The workers had a mean age of 48 ± 12 years with 28 ± 8 years on the job.

Results

Clinical examination: Findings on pulmonary examination were minimal. There were five cases of clubbing and four instances in which fine rales were heard.

Pulmonary function: Standard spirometry was performed on a Systems Research Laboratory screener. The measurements used were FVC/VC predicted, FEV_1/FVC, and, as a more sensitive measure of obstruction in small airways, the FEF_{25}/FVC ratio (FEF_{25} is the forced expiratory flow rate with 25% of the vital capacity remaining to be expired). The results were classified into either restrictive, obstructive, combined, or normal according to the schema given in Table VI; the results are detailed in Table VII. 29% had decreased vital capacity, the percentage increasing with age and most markedly after 20 years from onset on auto work (Table VIII). The differences are statistically significant $.05 > p > .02$.

Effect of smoking: Significantly more smokers and ex-smokers than non-smokers were obstructed (Table IX $X^2 = 8.48$ $.01 > p > .001$). (See note at end of the Table.) There was no significant difference, however, among smokers, non-smokers, and ex-smokers with respect to restriction ($X^2 = .01$, not significant).

TABLE VI

Classification of Pulmonary Function in Vehicular Maintenance Workers

	$FEV_1/FVC \geq .75$		$.59 < FEV_1/FVC < .75$*	$.59 \geq FEV_1/FVC$*
	$FEF_{25}/FVC \geq .30$	$FEF_{25}/FVC < .30$		
FVC/VC pred $\geq .80$	Normal	Obstructive	Obstructive	Obstructive
FVC/VC pred $= .70-.79$	Restrictive	Combined	Combined	Obstructive
FVC/VC pred $\leq .69$	Restrictive	Combined	Combined	Combined

* Note: all those with $FEV_1/FVC < .75$ also had $FEF_{25}/FVC < .30$

TABLE VII

Pulmonary Function Abnormalities in 87 Vehicular Maintenance Workers with over 10 Years Experience

	Number	Percent
Restrictive	14	16%
Obstructive	32	37%
Combined	11	13%
Any abnormalities	57	66%

TABLE VIII

Restriction of Pulmonary Function by Year of Onset of Work in 90 Vehicular Maintenance Workers

Total restrictive and combined	Abnormal					
	1956–65		1946–55		Before 1946	
	Yes	No	Yes	No	Yes	No
25	1 (6%)	15	14 (33%)	28	10 (34%)	19

For before 1956 compared with 1956 and after: $X^2 = 4.84 \quad .05 > p > .02$

TABLE IX

Relationship between Obstruction or Restriction and Smoking in Vehicular Maintenance Workers

	Number of Subjects	Current Smokers	Never Smoked	Ex-Smokers
Normal	31	10	13	8
Obstructive	31	15	5	11
Restrictive	14	3	6	5
Combined	11	5	1	5
Total	87	33	25	29

For restriction $X^2 = .01$ N.S. (smokers and ex-smokers compared with never smoked)

For obstruction $X^2 = 8.28 \quad .01 > p > .001$

Those in the combined group counted as being both obstructive and restrictive

The relationship between pulmonary function signs of restriction and abnormal x-rays was investigated. No significant association was found (Table X).

Chest x-ray: The chest x-rays were read twice by consensus reading, using at least three experienced readers, and categorized according to the ILO/U/C classification. An x-ray was read as abnormal if fine or moderately thin, irregular linear opacities were present (s or t $\geq$ 1/0) and/or pleural thickening was present (a-1 or more). The prevalence of abnormal readings is given in Table XI. Parenchymal abnormalities were present in 19 instances, nine 1/0, six 1/1, two 1/2 and two 2/2. X-ray findings consistent with asbestosis increased with time; such a trend is evident in Table XII. This trend is statistically significant.

TABLE X

Relationship between X-Ray Reading and Pulmonary Function Tests in 87 Vehicular Maintenance Workers

Pulmonary function	Number of Subjects	X-ray reading		Percent
Restriction	25	Abnormal*	5	20%
		Normal	20	80%
Obstruction	43	Abnormal*	15	35%
		Normal	28	65%
Normal	30	Abnormal*	8	27%
		Normal	22	73%

* parenchymal fibrosis and/or pleural thickening

For overall pattern $X^2 = 1.78 \quad .50 > p > .30$

For restriction alone $X^2 = 1.21 \quad .30 > p > .20$

For obstruction alone $X^2 = 1.50 \quad .30 > p > .20$

For obstruction and restriction compared to normal: $X^2 = .08 \quad .80 > p > .70$

TABLE XI

X-Ray Changes in 90 Vehicular Maintenance Workers with over 10 Years' Experience

	Number	Percent
Parenchymal fibrosis	18	20%
Pleural thickening or calcification	5	6%
Both parenchymal and pleural changes	1	1%
Any changes	24	27%

TABLE XII

X-Ray of Vehicular Maintenance Workers by Year of Onset of Work

Abnormal*					
1956–65		1946–55		Before 1946	
Yes	No	Yes	No	Yes	No
3 (18%)	14	10 (23%)	33	14 (47%)	16

* Parenchymal fibrosis (ILO/UC s, t $\geq$ 1/0) and/or pleural thickening

X^2 for before 1956 compared with 1956 and after: $X^2 = 5.95 \quad .02 > p > .01$

Discussion

The amount of asbestos brake material worn away each year is enormous, and even if only a small percentage is morphologically unaltered, then hundreds of thousands of kilograms remain. Fiber counts in various operations in brake maintenance work show wide variations. Certainly, they indicate that these operations need more systematic evaluation, both as to peak and time-weighted values. Peak values frequently exceed the 10 fiber/ml legal limit for peak values in the United States.

Much of the chrysotile is altered to other phases, which may be fibrous or granular. The biologic activity of these materials has not been studied. Detailed classification and quantification of these changes has not been reported. However, even the incomplete correlations presented above between optical fiber counts and electron microscopic volumes of morphologically intact chrysotile is evidence that fiber counts are useful measures of asbestos exposure in brake repair work.

Many of the vehicular maintenance workers examined showed signs of asbestosis. The prevalence both of chest x-ray changes and restrictive function results was significantly higher after 20 years of exposure than before, a result expected after occupational exposure to asbestos (14, 15).

Summary and Conclusion

Measurements were made by optical microscopy of asbestos levels during brake repair and maintenance work in New York City. Both time-weighted averages and peak levels showed significant asbestos exposure. Over four-fifths of the total number of chrysotile fibers in brake-drum dust were below 0.4μ in length and not visible by optical microscopy. Over one-quarter of a group of experienced vehicular maintenance workers examined had evidence of x-ray abnormalities consistent with asbestosis; one-quarter also had restrictive pulmonary function test findings. While this preliminary study was limited in scope and was restricted to volunteers, and its results cannot therefore readily be generalized to all brake maintenance workers in the United States, the findings suggest that asbestos disease will be present among such workers and that appropriate control measures should be urgently instituted.

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Portal Surgery and Liver Function: A New Look at an Old Problem*

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The treatment of portal hypertension for the prevention of bleeding esophageal varices is a surgical shunt. To date, no effective therapy has been devised which predictably decreases the long-term risk of recurrent bleeding other than an effective and patent venous shunt. Nonshunting surgery and nonoperative therapy have not been successful in reducing significantly the frequency of recurrent upper gastrointestinal, presumably variceal, bleeding.

There are only two types of shunts for the effective prevention or control of bleeding esophageal varices caused by portal hypertension: 1) portal decompression (total shunt); and 2) variceal decompression (selective shunt).

There are only two types of operative procedures for portal decompression (both of these shunts decompress also the esophago-gastric varices via the left gastric vein). One is decompression of the extrahepatic portal system only. This is the end-to-side portacaval shunt. The other is decompression of both the intra- and the extrahepatic portal system. The prototype of this is the side-to-side portacaval shunt. There are over a dozen modifications of the side-to-side portacaval shunt. The only difference in the hemodynamic effects of these various portal decompressive procedures is due to the size of the shunt. The size of the anastomosis, or graft, affects the volume of flow through the shunt and, therefore, the effectiveness of the shunt: a) The larger the shunt, the smaller is the frequency of its thrombosis and recurrent hemorrhage but the more common is recurrent, often incapacitating, encephalopathy. b) Conversely, the smaller the shunt, the greater is the frequency of thrombosis and recurrent hemorrhage but less common is the development of encephalopathy.

In contrast to total shunts that decompress the varices by decompressing the portal vein, a selective shunt decompresses the varices only. There are only two truly selective shunts. One is the distal splenorenal (Warren) shunt (1), which consists of two procedures: a) Distal, end-to-side splenorenal anastomosis. This directs blood flow from the varices through the short gastric and the numerous collateral veins via the spleen into the renal vein; and b) Portal-azygos disconnection: All communications between the varices and the portal vein are ligated to prevent portal flow through the varices into the newly created shunt (Fig. 1).

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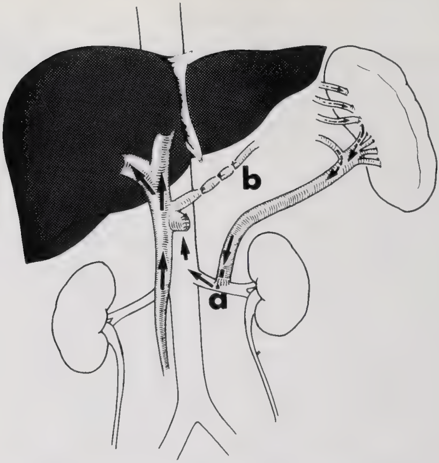

FIG. 1. Schematic Representation of the Selective Distal Splenorenal (Warren) Shunt. This operation consists of two surgical procedures: a) The construction of an anastomosis of the distal end of the splenic vein to the side of the left renal vein. Blood is drained from the varices through the short gastric veins and the spleen to the shunt; and b) Ligation of the left gastric vein to abolish all direct communication between the varices (which are decompressed by the splenorenal shunt) and the portal vein (in which the elevated pressure is maintained).

The second selective shunt is the anastomosis between the proximal end of the left gastric vein and the interior vena cava (2). This is also a truly selective shunt. The problem with this operation is that the long-term patency of the shunt is questionable. This is a low-flow shunt which involves the anastomosis of a small vein to the vena cava.

In the United States as well as in the rest of the world, the currently accepted treatment of bleeding esophageal varices due to portal hypertension is a portacaval shunt or one of its modifications. These shunts can be looked upon as central or total shunts. If the purpose of these operations was to prevent recurrent upper gastrointestinal bleeding, then these operations were successful. If the purpose of these operations was to increase the longevity of cirrhotic patients, then the success of total shunts was equivocal. Indeed, there was not even a significant decrease of mortality rates of shunted patients. Nevertheless, in certain patients, a total shunt was clearly a successful therapeutic intervention. There were no definite criteria, however, which permitted the selection of these "certain" patients before their shunt operation. If, however, the total shunts were to increase meaningful and productive life, then these operations were failures.

The authors are familiar with data based on four prospective controlled clinical trials for the evaluation of a total shunt (portacaval shunt). Two of these studies already have been reported in American literature (3, 4). These included 121 shunted and 102 control patients. The third study was from Clichy, France (5). The combination of these three studies included 303 patients. Up to 4 years of follow-up of the 152 shunted patients showed the mortality rates were almost identical to those of the 151 control patients (6). A fourth study is conducted by Reynolds* and his colleagues in Los Angeles. They found that shunted patients had probably lower mortality rates as compared to their controls. However, the different mortality rates were not significant at the 5% level of certainty. Nevertheless, even the apparent improvement of survival after portacaval shunt was negated by the high frequency of encephalopathy in these shunted patients.

These four prospective, controlled, clinical trials from academic institutions of well-deserved repute indicate, therefore, that productive and meaningful human existence was not prolonged by portal decompression—a total shunt—despite the fact that the shunt effectively reduced the frequency of recurrent upper gastrointestinal hemorrhage from whatever source.

Since the currently accepted practice for the treatment of this complication of cirrhosis did not have the anticipated outcome, then it is likely that the choice of therapy was based on wrong premises: 1) the selection of the patient for the operation was inappropriate, or 2) the selection of the operation for the patient was inappropriate, or, indeed, 3) both types of selections may have been inappropriate and were responsible for not improving encephalopathy-free survival rates.

The selection of patients for a shunt operation usually was based on clinical and laboratory evaluation of the patient. Child's criteria (Table I) can reliably predict the probability of survival after a shunt operation. Indeed, these criteria were good predictors of survival after surgery of any kind in cirrhotic patients, not only after a shunt operation. These criteria, however, did not predict the probability of encephalopathy in the surviving patients (7).

Altered consciousness, or coma, after a total shunt operation was usually a "nitrogenous" portal systemic encephalopathy. Therefore, the mechanism of encephalopathy after a total shunt probably was related, directly or indirectly, to abnormal metabolism of nitrogenous products, such as amino acids, amines, urea, ammonia, etc. It had been known for many years that a close association existed between the rise of blood ammonia and encephalopathy in a particular patient, although there was not consistently a good association between the means of blood ammonia concentrations in cirrhotic patients who had, versus those who did not have, encephalopathy. Nor were preoperative fasting blood ammonia or ammonium chloride tolerances (as they were performed and interpreted in the various institutions) valid predictors for the development of postshunt encephalopathy in cirrhotic patients. More recently, Fischer (8) suggested that accumulations of amines act as false neurotrans-

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* Personal communication

TABLE I

Laboratory and Clinical Criteria for Estimating Hepatic Reserve

Criteria	Good Risk A	Moderate Risk B	Poor Risk C
Serum bilirubin (mg/100 ml)	Below 2.0	2.0–3.0	Over 3.0
Serum albumin (gm/100 ml)	Over 3.5	3.0–3.5	Under 3.0
Ascites	None	Easily controlled	Not easily controlled
Encephalopathy	None	Minimal	Advanced
Nutrition	Excellent	Good	Poor

mitters and this is the mechanism of encephalopathy in cirrhotic patients. During the last weeks, a close association was demonstrated between portal-systemic encephalopathy in cirrhotic patients and the serum concentrations of two amines: octopamine (9) and tyramine (10).

During studies of nitrogen metabolism in cirrhotic patients a marked impairment of urea synthesis was identified as compared to normal individuals. In general, cirrhotic patients responded sluggishly in turning off or turning on their urea synthetic mechanism when the dietary nitrogen (protein) intake was increased or decreased, respectively. Furthermore, although fasting serum amino acid concentration in a group of cirrhotic patients was normal on a low protein diet, it became significantly elevated on high protein intake. The amino acid tolerances were abnormal following standard oral amino acid tolerance tests. These showed a "diabetic" type of curve regardless of the level of the corresponding fasting serum amino acid concentration (11).

One of the essential hepatic parenchymal functions is the synthesis of urea. Because of the demonstrated defects of nitrogen metabolism in cirrhotic patients, the measure of urea synthesis should be an appropriate hepatic function test in these patients. To evaluate the capacity of the human liver to synthesize urea, we increased the blood amino acid nitrogen concentrations over 14 mg/dl either by feeding large amounts of protein by mouth or by infusing L-amino acids intravenously. As the blood amino acid concentrations rose, the rate of urinary urea excretion increased and eventually reached a maximum both in normal volunteers and in cirrhotic patients. When urinary excretion of urea nitrogen could no longer be increased by either increasing the rate or prolonging the duration of amino acid infusions to the point of nausea, then the maximum rate of urinary urea-nitrogen excretion (MRUE) was reached as its excretion rate plateaued at a new high level. The MRUE was lower in cirrhotic patients than in the normals. During the period of MRUE, a new steady state of urea production was established. During this new steady state, the rate of urea synthesis was estimated by making appropriate measurements of the increased urea in total body water, of the urea hydrolized by ureases and, of course, excreted in the urine. This was the estimate of the maximum rate of urea synthesis (MRUS). Under well-defined conditions, the MRUS is a measure of hepatic functional capacity (12). The MRUS was reached when plasma amino acid nitrogen concentration exceeded 14 mg/dl. Under these conditions, normal individuals synthesized 65 ± 2.1 mg of urea nitrogen per hour per $Kg^{3/4}$. The reason for this unit is that the nonedematous body weight raised to the 3/4 power is the most closely related linear function of the metabolically active body mass (12).

The MRUS was significantly higher in normals than in cirrhotic patients. The MRUS was significantly higher in cirrhotic patients without encephalopathy than in those cirrhotics who had a history of one or more spontaneous episodes of (nitrogenous) encephalopathy. The MRUS in over 60 cirrhotic patients did not correlate with any of the conventionally used clinical laboratory hepatic tests; that is, with albumin, gamma globulin, SGOT, alkaline phosphatase, bilirubin, prothrombin time, etc. (12).

The MRUS, therefore, could be a measure of the ability of the cirrhotic patient to handle nitrogen and may be a predictor of such a patient's ability to tolerate shunt surgery of some type.

To evaluate the clinical value of a selective as compared to a total shunt and to explain some of the reasons for the failure of total shunts to prolong productive life after variceal hemorrhage, a prospective, controlled, clinical trial was designed. For the total shunt, a recently introduced and most popular variety of portal decompression was used: the H-graft mesocaval or mesorenal shunt as described by Drapanas et al (13). For the selective shunt the distal splenorenal shunt was used (1). For brevity the total H-graft interposition mesorenal shunt will be referred to as H-graft shunt and the selective distal splenorenal shunt will be referred to as Warren shunt.)

So far, 46 patients were accepted for randomization. Twenty-four were randomized for H-graft and 22 for Warren shunt. The age and sex distribution of the two groups of patients were similar. Eighteen of the 24 patients in the H-graft shunt group were patients from the private sector and 6 from the public (V.A. or county hospital) sector. Eleven of the 22 patients in the Warren shunt group were from the private sector and the remaining 11 from the public sector. None of the patients had ascites or active parenchymal necrosis (chronic active or alcoholic hepatitis). Although several of these patients did have either ascites or active liver disease or both in the past, these abnormalities responded to therapy. By the time of randomization and surgery, there was no detectable ascites on physical examination nor significant active parenchymal necrosis on liver biopsy. The operative mortality (none in each group) and the hospital mortality (1 in each group) were the same. The long-term mortality in the H-graft shunt (5 of 24) appeared larger than that after the Warren shunt (1 of 22), but this difference could have occurred by chance alone at $p > .05$. Encephalopathy developed more frequently after the H-graft (8 of 24) than after the Warren (1 of 22) shunt; a difference which was significant at the $p < .05$ level.

Before surgery the MRUS was the same in the H-graft group as in the Warren shunt group. The hepatic functional capacity, as measured by the MRUS, was depressed by the H-graft shunt. The decrease of the MRUS after the H-graft shunt was greater than that after the Warren procedure ($p < .05$). All patients who developed postshunt encephalopathy decreased their MRUS from the preoperative level. When the patients developed encephalopathy, their MRUS decreased to below 33. These patients had the lowest MRUS values in their respective groups. Those individuals who had MRUS over 45 before their shunt surgery did not decrease their postoperative MRUS low enough to fall below 33, the range where the risk of encephalopathy became great, nor did they develop clinically recognizable encephalopathy during their follow-up period, regardless of the type of their shunt.

These observations indicate that a selective decompression of varices did not impair hepatic functional capacity to the extent that a total shunt does. Selective shunt did not alter portal pressure and, presumably, portal flow. While the total shunt decreased portal pressure, it therefore must have decreased portal flow. This contention was documented in postoperative angiograms in these patients. In the H-graft group, either reversal of portal venous flow through the shunt or at least diversion of mesenteric flow through the shunt to the vena cava (hepatofugal) rather than up the portal vein to the liver (hepatopetal) in some patients. After the H-graft shunt, opacification of the portal vein was seen following injection of contrast material in the hepatic artery. In such patients simultaneous injection of celiac (hepatic) and superior mesenteric arteries can result in a misleading venous phase in which both the superior mesenteric and portal veins become opacified. However, the portal vein filled retrograde and could not be used as evidence for hepatic perfusion. Such erroneous interpretation could even pass editorial review (13). However, after the Warren shunt, no apparent alteration was demonstrable of mesenteric flow to the portal vein and to the liver. Wedged hepatic vein pressures were not affected by the Warren shunt.

Summary and Conclusion

Although the currently used criteria for the selection of patients for portal systemic shunt operation are effective predictors of postoperative mortality, these do not indicate the risk of postshunt encephalopathy. On the other hand, quantitative estimate of hepatic function by the maximum rate of urea synthesis (MRUS) is a good predictor of postshunt encephalopathy. Those patients who have near-normal MRUS have little risk of developing postshunt encephalopathy, but the risk is substantially greater for those patients whose MRUS is impaired.

Therefore, 1) the preshunt evaluation of patients should include a quantitative estimate of hepatic functional capacity. The conventional hepatic tests and clinical evaluation provide insufficient information to judge the risk of productive postoperative life. 2) The selective decompression of varices is an effective prophylaxis against recurrence of variceal bleeding. In contrast to a portal decompression (a total shunt), the selective shunt is associated with a significantly lower risk of posfoperative encephalopathy, probably because hepatic function is depressed by a total shunt.

The following patients are not candidates for the Warren shunt unless additional effective therapeutic measures become available in the future: a) Those who currently have ascites (because of excessive postoperative morbidity and mortality due to reaccumulation of ascites); b) those who have no significant portal venous flow to the liver (because even a total shunt will not decrease a nonexistent portal hepatopetal flow); and c) those in whom previous operations, trauma, or congenital abnormalities preclude the construction of the distal splenorenal shunt.

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Small-Cell Dysplasia and in Situ Carcinoma of the Breast: Incidence and Age Distribution in Patients Biopsied over a 29-Year Period

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Abstract

Review of 4025 breast biopsies taken during the years 1946–1974 inclusive at The Mount Sinai Hospital revealed 189 cases (4.7%) of small-cell dysplasia and in situ carcinoma. The largest number of patients was found in the decade 40–49. Next in order of frequency was the succeeding decade (50–59). During the period 1946–1967, when few sections were taken from each specimen, the incidence of these changes was 3% of all biopsies. From 1968 onward the number of blocks processed per specimen has been progressively increased, with a progressive increase in the detection of atypical changes—to a maximum of 14% during 1974. Dysplastic lesions are focal, and comprehensive sectioning of specimens is essential if they are not to be overlooked.

We have previously recorded our experience with regard to the incidence of small-cell dysplasia and in situ carcinoma in some 2600 breast biopsies taken during the years 1946–1967 at The Mount Sinai Hospital (1). This report will amplify that experience.

Material

Biopsies taken during the years 1946–1974 inclusive have been reviewed. The 4025 biopsies examined consisted mainly of excisions for fibrocystic disease, but tissues removed during mammoplasty, and breast tissue adjacent to fibroadenomas, when present, have been included. Specimens containing invasive carcinomas have been excluded.

Method

During the period 1946–1967 no more than 1 or 2 sections were usually taken from each breast biopsy. Subsequent to that time the number of blocks studied has been progressively increased. Initially a minimum of 3 blocks was taken if the patient was over the age of 40 years. This number was later increased to a minimum of 5 blocks in all cases, irrespective of age. If the material submitted by the surgeon was insufficient for the provision of 5 blocks, the entire specimen was embedded. Where some index of suspicion existed (e.g., contralateral invasive carcinoma, or atypical changes in the initial sections) we have had no hesitation in processing 10 or more blocks. Radiology of specimens has not been employed.

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Pathology

The criteria previously employed for the categorization of small-cell lesions (1, 2) have been generally adhered to. However, whereas our earlier observations (1, 2) included both solid and cribriform proliferations, the present report pertains solely to the solid form. In an attempt to attain the greatest possible uniformity, cribriform and papillary lesions have been excluded. Large-cell lesions have, similarly, not been included. All of the cases previously reported (1, 2) have been reviewed. Some have been excluded because it was felt on reexamination that the changes were too marginal to warrant retention; and some have been reclassified.

Division of the small-cell lesions into categories (1, 2) has been retained. Thus the lesions have been subdivided, according to the degree of proliferation, into 3 Grades—Grade I (atypical dysplasia), Grade II (indeterminate), and Grade III (frank in situ small-cell carcinoma of ducts and/or lobules). The structural alterations observed in the various grades have previously been described in detail (1, 2) and will not be repeated. They are, however, illustrated for reference in Figures 1–4.

Findings

The total incidence of small-cell lesions (Table I) over the 29-year period amounted to 189 instances out of 4025 biopsies (4.7%), comprising (Table II)

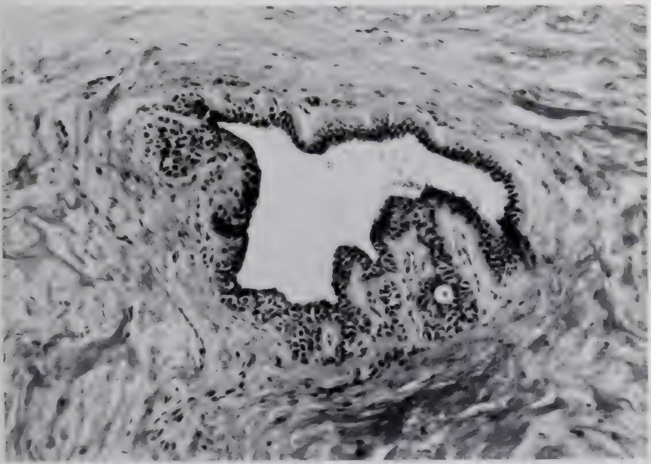

FIG. 1. Grade I lesion. Low-power micrograph showing basal proliferation of small cells (H&E x 80).

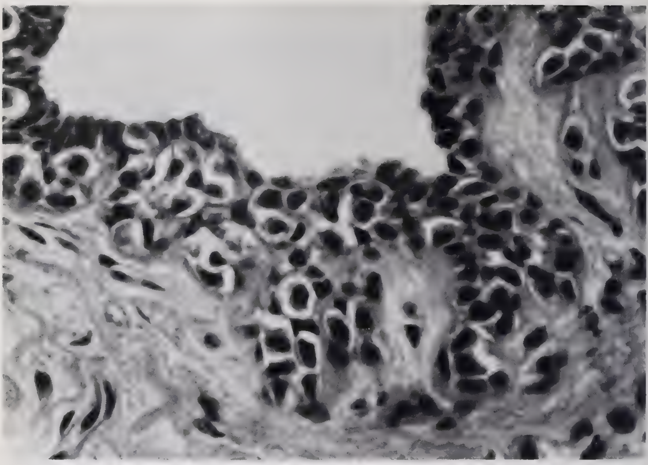

FIG. 2. Grade I lesion. High-power micrograph of preceding showing proliferation of polygonal small cells within the basal layer (H&E x 320).

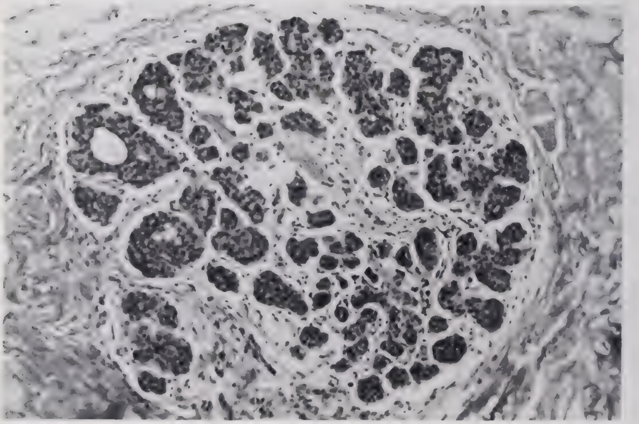

FIG. 3. Grade II lesion. The acini are crowded by masses of small cells (H&E x 80).

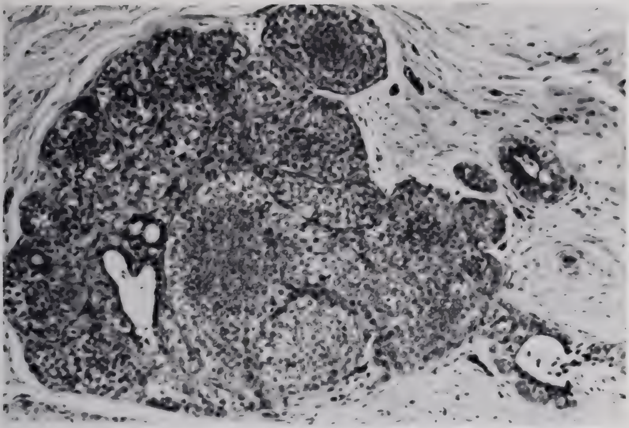

FIG. 4. Grade III lesion. The acini are grossly distended and globular. They are filled by homogeneous masses of small cells (H&E x 80).

TABLE I

Incidence of Small-Cell Lesions during the Years 1946–1974

No. biopsies	No. lesions	% Lesions
4025	189	4.7

TABLE II

Grading of Small-Cell Lesions (1946–1974)

Grade	No. cases	%
I	100	53
II	45	24
III	44	23

100 examples in Grade I (53%), 45 in Grade II (24%), and 44 in Grade III (23%). Yearly figures are not available for the period 1946–1967, but the incidence during this time was 3% (72 out of a total of 2594 biopsies). Annual figures are, however, available for the years 1968–1974 (Table III). It is seen that the number of small-cell lesions has progressively increased, with a final average during 1974 of 14%. The distribution of the lesions within the 3 grades is shown in Table IV. The number of Grade I lesions has generally exceeded that of either Grade II or Grade III proliferations.

The age distribution of the patients is recorded in Table V. An essentially similar pattern is observed in all 3 grades, as well as in an analysis of the total

TABLE III

Yearly Incidence of Small-Cell Lesions (1946–1974)

Year(s)	No. lesions	No. biopsies	% Lesions
1946–1967	72	2594	3
1968	11	219	5
1969	11	152	7
1970	13	174	7
1971	10	193	5
1972	12	203	6
1973	21	219	9
1974	39	272	14

TABLE IV

Incidence of Small-Cell Lesions According to Grades (1946–1974)

Year(s)	Grade I	Grade II	Grade III	Total
1946–1967	43	12	17	72
1968	6	4	1	11
1969	5	4	2	11
1970	3	5	5	13
1971	6	2	2	10
1972	6	2	4	12
1973	12	6	3	21
1974	19	10	10	39
Total	100	45	44	189

TABLE V

Age Distribution of Patients per Decade

Years of Age	20–29	30–39	40–49	50–59	60–69	70–79	80–89
Grade I	1(1%)	10 (10%)	42 (42%)	31 (31%)	12 (12%)	1 (1%)	3 (3%)
Grade II	2 (4%)	3 (7%)	24 (53%)	14 (31%)	1 (2%)	1 (2%)	0
Grade III	0	6 (14%)	20 (47%)	10 (21%)	6 (14%)	2 (5%)	0
Total	3 (2%)	19 (10%)	86 (45%)	55 (29%)	19 (10%)	4 (2%)	3 (2%)

group. The largest number of patients falls into the decade 40–49. Next in order of frequency is the succeeding decade (50–59). A substantially smaller number of cases is found in the decades preceding and succeeding this 20-year span (40–59), with the smallest numbers at the extremes of age (decades 20–29 and 80–89 respectively).

Comment

The actual incidence per decade in the total patient body of 4025 is unknown, for an analysis of the total group according to age is not available. Analysis per decade exists only with regard to the affected patients. In view, however, of the large number of biopsies investigated and the prolonged period of time under study, it is reasonable to surmise that the frequency per decade observed in the affected group does reflect the actual frequency per decade in this particular population (of 4025 patients), and is not merely a function of variation in exposure to biopsy within the differing age groups.

Dysplastic lesions are focal. Consequently, detection of these changes is dependent in considerable measure upon the extent of tissue surveyed. Not infrequently, lesions have been detected in but 1 or 2 out of 10 or more sections.

We have no reason to believe that the increase in number of lesions observed in more recent years represents an actual increase in incidence of the disease. We consider that the increase reflects merely improved detection resulting from greater familiarity with the morphology of the early changes, and from more comprehensive sampling of the tissue.

Radiology of specimens cannot, in our opinion, supplant comprehensive sectioning. Although we have not tabulated the incidence of calcifications within the lesions in this series, it may be stated that a substantial number of these lesions, particularly in the early stages, do not exhibit calcification.

It may be noted that approximately one half of the cases were classified as Grade I, with Grades II and III together comprising the balance. This distribution is difficult to interpret, but may suggest that some 50% of the initial lesions will progress beyond that stage.

The incidence of small-cell lesions in breast biopsies is appreciable—averaging, during the final year of our study, a figure of 1 out of 7. The advanced, florid lesion presents an imposing picture and is unlikely to escape attention. By contrast, the early basal mobilization of small cells seen in the Grade I lesion (Figs. 1, 2) is inconspicuous and readily overlooked. Even if observed, the innocuous aspect of the process may mask the serious potential of the lesion. Multiple sections are essential if the changes are to be detected, and familiarity with the morphology of the initial stages is of equal importance if their significance is to be appreciated.

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Atypical Small-Cell Dysplasia and in Situ Carcinoma of the Breast: Incidence of Subsequent Invasive Carcinoma

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Abstract

Review of 4025 breast biopsies taken during the 29-year period 1946–1974 inclusive at The Mount Sinai Hospital has revealed 189 cases of atypical small-cell dysplasia and in situ carcinoma, an average incidence of 4.7%. Lesions have been classified, according to the degree of severity, into 3 grades—atypical dysplasia, indeterminate, and frank in situ carcinoma. Long-term observation indicates that these patients have a higher incidence of subsequent invasive carcinoma in the ipsilateral breast than would be expected in the general population, but numbers are not sufficient for a comparison between the 3 grades. Until further data accumulate it is our policy to recommend mastectomy for the florid, frank in situ carcinomas, while adopting a more conservative attitude toward alterations of lesser magnitude.

We have previously recorded our experience with atypical small-cell dysplasia and in situ carcinoma of the breast diagnosed at The Mount Sinai Hospital during the period 1946–1967 (1, 2). The present report will amplify that experience, and will comprise data derived from lesions diagnosed between the years 1946–1974 inclusive.

Material: Biopsies taken during the years 1946–1974 inclusive have been reviewed. The 4025 biopsies examined consisted mainly of excisions for fibrocystic disease, but tissues removed during mammoplasty, and breast tissue adjacent to fibroadenomas, when present, have been included. Specimens containing invasive carcinomas have been excluded.

Pathology: The criteria previously employed for the categorization of small-cell lesions (1, 2) have been generally adhered to. However, whereas our earlier observations (1, 2) included both solid and cribriform proliferations, the present report pertains solely to the solid form. In an attempt to attain the greatest possible uniformity, cribriform and papillary lesions have been excluded. Large-cell lesions have, similarly, not been included. All of the cases previously reported on (1, 2) have been reviewed. Some have been excluded, for it was felt, on reexamination, that the changes were too marginal to warrant retention; others have been reclassified.

Division of the small-cell lesions into categories (1, 2) has been retained. Thus the lesions have been subdivided, according to the degree of proliferation, into 3 Grades—Grade I (atypical dysplasia), Grade II (indeterminate), and Grade III (frank in situ small-cell carcinoma of ducts and/or lobules). The structural alterations observed in the various grades have previously been

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described in detail (1, 2) and will not be repeated. They are, however, illustrated for reference in the figures (1–3).

It is appropriate to emphasize again our contention that the geographic distribution of the lesions within the mammary tree is not specific. Thus the atypical small-cell proliferation may affect either ducts or lobules, independently or in combination. There is no regional specificity, and for this reason we prefer the designation 'small-cell dysplasia or in situ carcinoma' to that of 'lobular dysplasia or lobular carcinoma in situ.' The essential feature is a uniform proliferation of small cells, growing in solid sheets, and this process may be intraductal, intralobular, or both.

The incidence of small-cell lesions during the 29-year period under consideration has been discussed elsewhere (3). Suffice it to observe that 189 cases were detected out of the 4025 biopsies examined (4.7%).

Incidence of Subsequent Ipsilateral Invasive Carcinoma

Follow-up is maintained on an annual basis, and is continued for 20 years after detection of the initial lesion. Table I presents data pertaining to the development of subsequent ipsilateral invasive carcinoma. These data pertain solely to patients subjected to no more than biopsy at the original procedure. The 20-year period is subdivided into 5-year increments. The population at risk for a particular period comprises those patients who have safely tra-

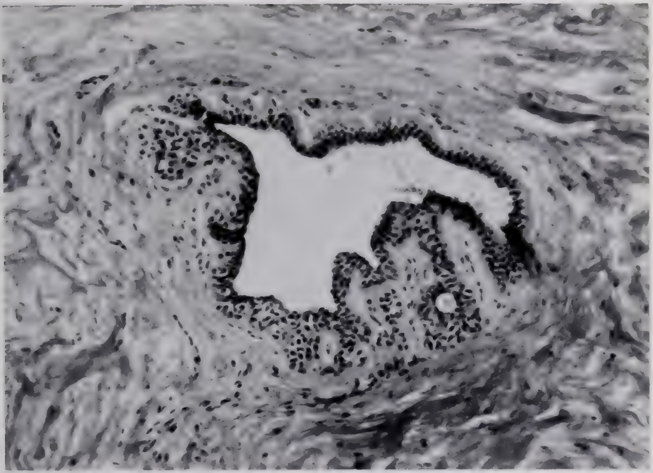

FIG. 1. Grade I lesion. Low-power micrograph showing basal proliferation of small cells (H&E x 80).

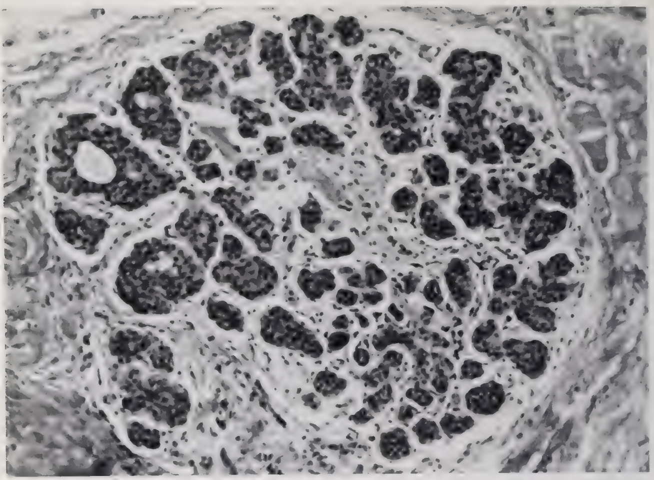

FIG. 2. Grade II lesion. The acini are crowded by masses of small cells (H&E x 80).

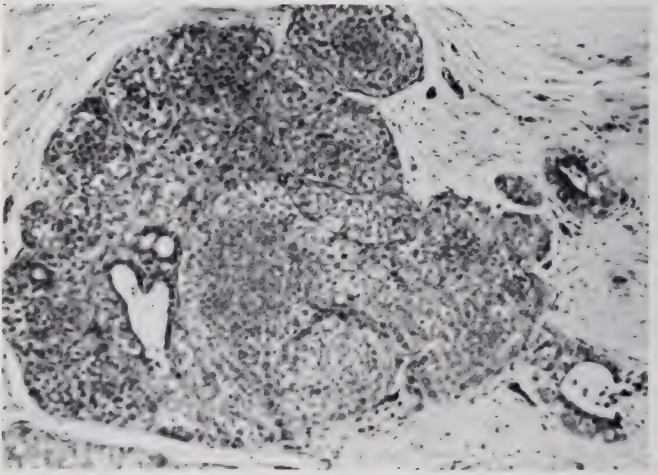

FIG. 3. Grade III lesion. The acini are grossly distended and globular. They are filled by homogeneous masses of small cells (H&E x 80).

TABLE I

Incidence of Ipsilateral Invasive Carcinoma

Grade	Year	Population at risk	No. carcinoma	% Carcinoma
I	5	49	2	4
I	10	32	4	12
I	15	25	5	20
I	20	16	6	38
II	5	17	0	0
II	10	7	0	0
II	15	5	1	20
II	20	2	1	50
III	5	18	2	11
III	10	13	3	23
III	15	12	6	50
III	20	10	6	60
I, II, III	5	83	4	5
I, II, III	10	52	7	13
I, II, III	15	42	12	29
I, II, III	20	28	13	46

TABLE II

Incidence of Prior Contralateral Mastectomy

Grade	No. cases	No. mastectomy	% Mastectomy
I	99	8	8
II	42	3	7
III	40	6	15
I, II, III	181	17	9

versed the period under consideration without developing infiltration, and those who have, at any time during that period, returned with an ipsilateral invasive carcinoma. Figures are presented both for the total group, and for the 3 categories previously defined. To date 13 invasive tumors have developed in the entire series. An analysis of the time interval to infiltration (4) has been presented elsewhere, as has a description of the subsequently appearing invasive carcinomas (5). It may, however, be noted that the majority of the subsequently appearing invasive lesions manifested during the period 6-15 years after the initial biopsy. Fifty percent of these invasive tumors were of small-cell type, the balance being composed of large cells, or a mixture of small and large cells.

Carcinoma in the Contralateral Breast

A substantial number of patients in whom dysplastic small-cell lesions were detected on the one side, had previously undergone mastectomy for contralateral invasive carcinoma, and a further group manifested simultaneous contralateral infiltrating lesions at the time of biopsy. Tables II, III, and IV present data with regard to the incidence of prior and simultaneous contralateral invasive carcinomas. These patients are excluded from consideration in

TABLE III

Incidence of Simultaneous Contralateral Mastectomy

Grade	No. cases	No. mastectomy	% Mastectomy
I	99	7	7
II	42	3	7
III	40	2	5
I, II, III	181	12	7

TABLE IV

Combined Incidence of Prior and Simultaneous Contralateral Mastectomy

Grade	No. cases	No. mastectomy	% Mastectomy
I	99	15	15
II	42	6	14
III	40	8	20
I, II, III	181	29	16

TABLE V

Incidence of Contralateral Invasive Carcinoma

Grade	Year	Population at risk	No. carcinoma	% Carcinoma
I	5	46	0	0
I	10	30	0	0
I	15	23	2	9
I	20	14	2	14
II	5	16	0	0
II	10	6	0	0
II	15	5	0	0
II	20	1	0	0
III	5	17	2	12
III	10	12	3	25
III	15	10	3	30
III	20	7	3	43
I, II, III	5	79	2	3
I, II, III	10	48	3	6
I, II, III	15	38	5	13
I, II, III	20	22	5	23

Table V, which presents data relating to the incidence of subsequently appearing invasive tumors in the contralateral breast. The format is the same as that adopted for the ipsilateral breast.

Comment

We have recorded our findings for each of the 3 grades into which we have subdivided the general body of small-cell lesions, for it was felt that this information would be of interest. It should be observed, however, that the numbers available are not sufficient for a statistically significant comparison between the grades. Attention must, therefore, be directed mainly towards the figures available for the total grouping.

The difficulties of evaluation are considerable. The total number of cases available for follow-up is small. A prolonged period of observation is mandatory, and figures pertaining to 5- or even 10-year periods are virtually worthless, for many tumors develop after the 10-year period has passed. A diagnosis of frank in situ carcinoma (Grade III) is frequently followed by immediate mastectomy, reducing even further the small number of patients suitable for inclusion in this important category.

These difficulties notwithstanding, the data available do appear to indicate a significantly greater incidence of invasive carcinoma in the ipsilateral breast, when the sample available for the 15-year period is compared to the usually stated incidence of some 5% in the general population. Even this realization does not, however, resolve our dilemma, for it is not possible to justify mastectomy where the early lesions alone are detected. There can be no escape from this problem until sufficient numbers have accumulated to provide a valid comparison between the early and advanced lesions. It is traditional to assume that the initial basal proliferation is less likely to invade than the florid, frank, in situ carcinoma, but this belief must, for the present, remain no more than an assumption.

We have hitherto retained a 3-category system of classification for morphologic convenience. The Grade II or indeterminate grouping provides relief from the difficult and tendentious task of deciding whether many lesions should be classified as severe dysplasia or as in situ carcinoma. It is of little moment, however, whether a 2- or 3-category system is adopted, provided that long-term data accumulate which will indicate whether a difference in prognosis exists between the initial and the advanced lesions.

Until such information is available we shall continue, in the main, to recommend simple mastectomy for the florid, extensive, Grade III lesion (frank in situ carcinoma), while urging a conservative approach to the alterations of lesser magnitude. The empiricism of this approach, must, however, be conceded—for it is not impossible that the morphologically less advanced 'dysplasia' may possess a biologic capability for infiltration equal to, or even greater than, that of the 'in situ carcinoma.'

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In Vivo Effects of Concanavalin A. II. Distribution, Histopathologic Changes, and Toxicity

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Abstract

The in vivo tissue distribution and histopathologic effects of Concanavalin were studied in CBA mice. After intravenous administration of I^{125}-labeled Con A, high concentrations were rapidly reached in the blood, liver, lymph nodes, and spleen. Lower and delayed peak concentrations followed subcutaneous and intraperitoneal administration. Lymphocytoxicity, especially marked in the paracortical areas of the lymph nodes, perifollicular protein precipitation in the spleen, and midzonal liver cell injury were prominent histologic changes. The thymus did not show any significant change. Very high SGOT levels and a fall in blood sugar levels confirmed liver cell toxicity. Administration of alpha methyl mannose or alpha methyl glucose before or with Concanavalin A did not appreciably alter its effects. The immunosuppressive activity and toxicity of Con A might be explained by its respective actions on peripheral lymphocytes and on the liver.

Introduction

In a previous study (1), it was shown that Concanavalin A (Con A) administered intravenously, was capable of prolonging survival of murine skin allografts between strains of mice differing at the H-2 histocompatibility locus. Although immunosuppression by Con A is a consequence of its direct action upon lymphocytes, Con A may interact with receptors present on a wide variety of mammalian cells. Its action on nonlymphoid cells may have accounted for its toxic side effects which limited the dose and consequently the obtainable immunosuppression.

In order to determine the relative affinity of Con A for various tissues, the ensuing studies of the in vivo distribution of this material and this histologic and biochemical changes following its administration were performed.

Methods and Materials

Mice: Male inbred mice of the CBA/J (CBA) and A/J (A) strain, 2- to 3-months-old, were obtained from the Jackson Laboratories, Bar Harbor, Maine.

Skin grafts: A-strain tail skin was grafted onto CBA recipients according to the principles outlined by Billingham and Medawar (2).

Plaster casts were removed on day 7 after grafting and the end point of graft

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survival was taken as the day when no viable epithelium was macroscopically visible. Intact skin grafts from Con A-treated animals were examined histologically 12 and 17 days following grafting.

Concanavalin A (Con A): Con A, lyophilized in saline (Calbiochem) was diluted to 10 mg/ml in distilled water and further diluted for injection in Minimal Eagle's Medium (MEM, Microbiological Associates, Bethesda, Maryland). Con A was administered in various routes, dosages, and time schedules as described below. In some experiments Con A was given 5 minutes before or after the administration of an equal volume of a 5% solution of alpha methyl glucose or alpha methyl mannose. In other experients these saccharides were mixed with Con A in equal volumes before administration.

Con A was trace labeled with I^{125} by the chloramine-T method using 500 microcuries NaI^{125} (Sp. Act. 117mC-mg, Amersham Searle Corp. Des Plaines, Illinois), 5 mg Con A, 0.05 mg Chloramine T, and 0.01 mg potassium iodide in 1.0 ml of 0.15 M pH 7.6 phosphate buffer. After labeling at room temperature had taken place for 30 minutes, sodium metabisulfite, 0.2 mg, was added, and labeled protein separated by passage through 0.9 x 50 cm column of Sephadex G-25.

Ninety-five percent of the radioactivity in the protein peak could be precipitated by 10% TCA or by 1% shellfish glycogen, Type II (Sigma Chemical Co.).

Distribution: Four hundred μg of the I^{125}-labeled Con A in 0.5 ml of Minimal Essential Medium was injected intravenously, subcutaneously, or intraperitoneally. Two mice from each of these groups were sacrificed at 5 minutes, and 1, 5 and 24 hours after injection. The blood (0.5 ml), peripheral nodes (axillary, brachial, and inguinal), mesenteric nodes, spleen, liver, kidney, lung, and thymus from each mouse were weighed and separately placed into 2 cc test tubes. The blood was centrifuged at 1,500 rpm, and the serum and cellular elements were weighed and placed in separate tubes. Each tube was counted for radioactivity for 10 minutes in Packard autogamma scintillation counter and counts were expressed per milligram of weight.

Histology: Mice were sacrificed by cervical dislocation at variable periods after the injection of Con A in various routes and doses. The peripheral lymph nodes, mesenteric nodes, Peyer's patches, spleen, kidney, thymus, lungs, surviving grafted tail skin, and skin injected intradermally with Con A were fixed in 10% formalin for histologic studies. Sections were stained with hematoxylin and eosin and were read without prior knowledge of the treatment given.

Biochemical studies: Serum glutamic oxaloacetic acid (SGOT), blood urea nitrogen (BUN), and blood sugar determinations were made using a micromethod from blood obtained by heart puncture at various periods after intravenous Con A (400 μg).

Results

Distribution (Figs. 1–3): Following intravenous administration, peak concentrations of Con A were rapidly reached in most tissues. Con A was rapidly

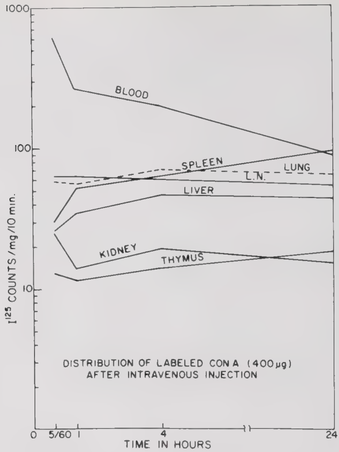

FIG. 1. Distribution of labeled Con A (400 μg) after intravenous injection.

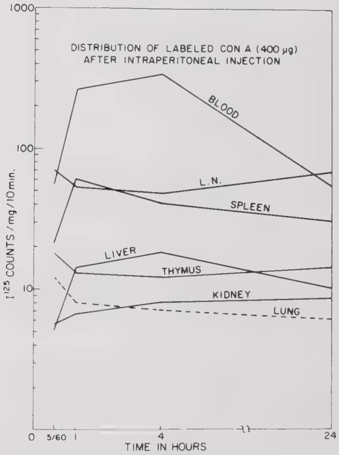

FIG. 2. Distribution of labeled Con A (400 μg) after intraperitoneal injection.

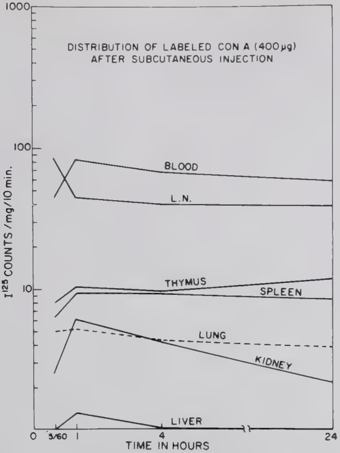

FIG. 3. Distribution of labeled Con A (400 μg) after subcutaneous injection.

cleared from the peripheral blood so that very little of the circulating serum or RBC contained Con A 24 hours after administration. In the spleen, however, the concentration of Con A was initially low and reached peak levels only at 24 hours. With intraperitoneal and subcutaneous injections, lower and delayed peak tissue concentrations were reached. Despite the route of administration, there was a relatively high concentration of Con A in the lymph nodes. Subcutaneous injection especially enhanced the accumulation in the peripheral nodes. The thymus did not selectively concentrate in Con A and the counts obtained here probably represented that of its contained peripheral blood.

Histology: At 1 and 4 hours after intravenous administration of Con A (500 μg), there was gross congestion of the peripheral and mesenteric nodes. Every Peyer's patch showed 1 or more petechiae. Considerable lymphadenopathy ensued by 24 hours. Histologically the lymph nodes at 4 hours showed congestion and reticulum cell hyperplasia; a few areas showed clumps of pyknotic lymphocytes. By 24 hours a characteristic pattern was seen in the lymph nodes, consisting of accumulations of pyknotic nuclear debris with active phagocytosis, primarily confined to paracortical areas (Fig. 4). Similar findings were present in Peyer's patches. There was no unusual activity in the germinal centers. Up to 12 days later, the nodes were hyperplastic and still showed some signs of phagocytosis of cellular debris.

The spleen appeared markedly congested at 1 hour, and was consistently

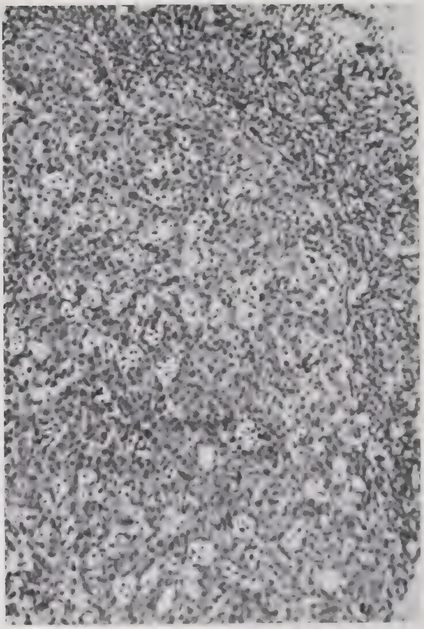

FIG. 4. Peyer's patch 24 hours after intravenous injection of 500 μg Con A. Note the numerous cell fragments within follicular sinusoids (H & E × 160).

enlarged and hemorrhagic at 4 hours after Con A (500 μg). By 24 hours the spleen size had returned almost to normal, and by 12 days the spleen was normal in size. Histologically there was diffuse hemorrhage in the red pulp at 4 hours, with dead lymphocytes clearly evident at 24 hours. A characteristic finding which appeared to be specific for Con A was protein precipitation in the spleen at the junction between the red and white pulp (Fig. 5). The deposited protein appeared not to have attracted an inflammatory infiltrate or resulted in any necrosis. The precipitation was still evident at 24 hours but by 12 days it had usually cleared.

One or 4 hours after injection, the liver appeared congested but at 20 to 24 hours, pale, sometimes with necrotic areas. The early lesion on histology showed congestion, cloudy swelling of cells, and cellular infiltrate. Between 20 to 24 hours, mid-zonal necrosis (Fig. 6), sometimes with lipoid regeneration, was a characteristic finding. Those animals who went on to survive showed a histologically normal liver at 12 and 17 days.

The thymus, kidney, lungs were grossly and microscopically normal. This was despite the high concentration of Con A in the lungs with intravenous administration.

The gross appearance and histologic changes in the above organs appeared earlier or more extensive when Con A was given in doses greater than 500 μg per mouse. Also the administration of alpha methyl glucose or alpha methyl mannose, before, with, or after Con A did not appear to significantly alter these findings or change the mortality.

Skin lesions: The earliest noticeable lesion at the site of intradermal injection of Con A was loss of hair. This was followed by reddening over the area, which then went on to subside or to ulcerate, depending on the dose. The earliest microscopic finding with small doses of Con A (0.3 mg) was a heavy mononuclear infiltration. By 6 days polymorphonuclear leukocytes were seen; and by the 10th day, the skin had returned to normal. With larger doses (0.6 mg) an acute inflammatory response was present at 6 days. With still larger

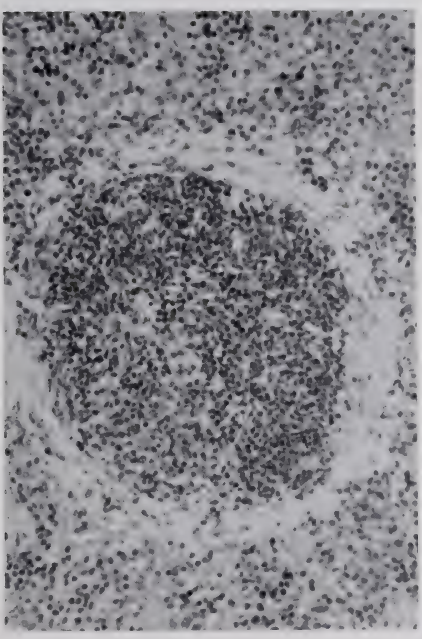

FIG. 5. High-power view of splenic follicles 24 hours after intravenous injection of 500 μg Con A. Note the deposition of proteinaceous material encircling the splenic follicle. Similar deposits of eosinophilic material were found surrounding all of the splenic follicles (H & E × 160).

doses (greater than 1.25 mg) necrosis of skin was invariable. The extent of necrosis was dose-dependent, and appeared early with high doses. With necrosis following high doses of Con A, there was a surprising lack of cellular infiltrate around necrotic areas.

Skin grafts: A moderate mononuclear infiltrate surrounded 12-day-old viable skin grafts. Heavy infiltrates of lymphocytes and plasma cells were seen in skin grafts surviving 17 days. Skin grafts usually reject at about this time with this dose of Con A (500 μg) and this probably represented early rejection.

Biochemical changes (Table I): Following 400 μg of Con A given intravenously, there was almost a 20-fold rise in the SGOT levels at the end of 24 hours. The BUN did not change significantly. The blood sugar fell at 24 hours to half the normal size.

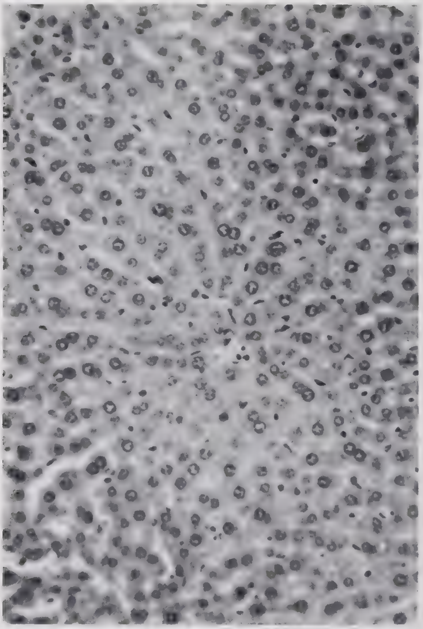

FIG. 6. High-power view of hepatic lobule 24 hours after intravenous injestion of 400 μg Con A showing collapse around the central vein with dissolution of live cells and nuclear pyknosis. Note the absence of any infiltration (H & E × 160).

TABLE I

Biochemical Changes[a] Following Con A (400 μg I.V.) Administration

Time after injection (No. of mice)	5 Minutes	1 Hour	4 Hours	24 Hours	Normal (No Con A)
SGOT (4)	60	95	230	2150	115
BUN (4)	34	31	34	23	29
Blood Sugar (4)	208	136	173	114	226

[a] Each result represents the mean of 4 determinations.

Discussion

These studies indicate that Con A administered in vivo is distributed widely and may interact with a variety of cells and tissues. There was some correlation between the concentration of Con A in various organs and the extent of histologic and biochemical changes. Thus, the spleen, liver, and lymph nodes showed both high concentrations of Con A and extensive gross and histologic injury while the thymus, kidney, and lungs showed low concentrations of Con A and no significant histologic injury.

Con A may accumulate in the reticuloendothelial organs by virtue of its ability to precipitate serum proteins (3–5) and to agglutinate red blood cells (6). Such denatured proteins or red blood cells may be filtered from the circulation in the liver and spleen. The presence of red cell engorgement and protein precipitates in the perifollicular areas of the spleen after Con A administration supports this view. The histologic changes in these organs may be secondary to this function rather than to a direct affinity for Con A.

Con A may also be directly cytotoxic. Cytotoxicity was a prominent finding in the lymph nodes, spleen and liver. The presence of extensive necrosis at the skin site of injection of Con A further supports such a mechanism. It has been suggested that Con A causes local effects by complement-mediated vascular damage (7). In our laboratory, however, Con A did not synergize with complement in promoting the in vitro cytotoxic action of rabbit antilymphocyte antiserum on mouse lymphocytes, and in higher doses inhibited the action of complement (Taub et al, to be published).

There are a number of similarities between the action of Con A and the action of heterologous or homologous antiserum to lymphocytes (ALS). Like antiserum (8), Con A administered in vivo preserves the thymic architecture but acts on peripheral lymphoid organs, conspicuously in the areas ordinarily populated by thymus-derived recirculating lymphocytes. Con A, like ALS, is more effective against skin allograft rejection than against humoral immunity (1). Both Con A (9–11) and heterologous (12–14) and isologous (15) ALS are known to cause blast transformation of lymphocytes in vitro. Although Con A unlike ALS possesses no intrinsic specificity towards lymphocytes, the net result of the in vivo Con A administration appeared to be lymphoid depletion because of the particular vulnerability of these cells.

The presence of numerous pyknotic cells in the lymph node paracortical areas after Con A treatment suggests that the recirculating lymphocytes may be especially vulnerable to the effects of Con A. Alternatively, recirculating lymphocytes may carry more surface receptor sites for Con A than bone marrow-derived lymphocytes. Con A did not concentrate significantly in the thymus, despite its abundance of lymphocytes. This suggests either that there are fewer available binding sites in the thymus or that there is a specific thymic barrier to the penetration of Con A as there is for ALS (16).

Both Con A (1) and homologous antiserum (15) are weakly immunosuppressive. The strong immunosuppressive action of heterologous antiserum is probably related to marked specificity against target cells.

Alpha-methyl glucose and alpha methyl mannose can react with Con A (17) and inactivate some of its in vitro actions (Taub et al, to be published). Injection of these sugar residues before, with or after Con A did not alter the mortality or histologic findings nor did not have any effect on allograft rejection. It is likely that Con A binds to the surfaces of cells more avidly than it binds to these haptens.

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Endocarditis and Osteomyelitis Caused by Aerococcus Viridans

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Abstract

Aerococcus viridans, though easily demonstrable in the environment, has infrequently been described as a human pathogen, perhaps because of similarities to group D and alpha-hemolytic streptococci. In this case, bacterial endocarditis on a prolapsed mitral valve and simultaneous back pain and osteomyelitis of the spine caused by Aerococcus viridans is documented. Biochemical and morphologic characteristics of the organism resembled those of streptococci of the Viridans group and the enterococci.

Introduction

Gram-positive cocci of the genus Aerococcus are usually considered part of the transient flora in the air of occupied rooms (1), and little has been written about their role as human pathogens. However, serious infections caused by members of this genus have occurred. In 1967, Colman (2) reported the isolation of ten strains of Aerococcus-like gram-positive cocci, four from blood cultures of patients with endocarditis, three from patients with urinary tract infections, and one each from an empyema, mouth area, and infected wound. The distribution of Aerococcus viridans is ubiquitous; however, the sparse documentation of this organism from human clinical infections may very well be due to its microbiologic similarity to alpha-hemolytic streptococci, and enterococci with which Aerococcus may easily be confused.

Subacute bacterial endocarditis is a disease frequently considered in the setting of fever, heart murmur, embolic phenomena, Osler's nodes, splinter and retinal hemorrhages. Less commonly recognized is the fact that back pain may dominate the clinical presentation (3).

Presented is a case of bacterial endocarditis accompanied by simultaneous back pain and osteomyelitis caused by Aerococcus viridans.

Case Report

A 54-year-old man was admitted to The Mount Sinai Hospital complaining of fever and back pain. Although informed of a heart murmur at various physical examinations since age 19, he had been asymptomatic and in excellent general health throughout his life. Four months prior to admission, he noted the insidious onset of a sharp low back pain, aggravated on exertion and persisting until the time of admission. During the two weeks before admission, he noted nightly sweats but denied fever or chills. Two days before admission, his private physician noted a systolic murmur at the apex, pain on straight leg-raising of either leg, and a temperature of 101°F, for which he was admitted for evaluation.

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On admission, oral temperature was 103°F but the patient appeared well nourished and in no distress. The left ventricular cardiac impulse was slightly diffuse and a III/IV late systolic murmur ending at the second heart sound was heard at the apex and radiated to the axilla upon standing. No clicks or gallops were present. The lordotic curve was straightened and there was pain on straight leg-raising to 30°.

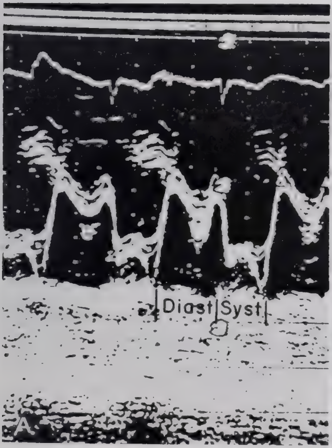
Pertinent laboratory data included erythrocyte sedimentation rate 92mm/hr; hemoglobin 10.6 g/100 ml; leukocyte count 14,200/mm^3 (segmented forms 75%, bands 5%, lymphocytes 14%, monocytes 6%); gamma globulin 1.66%; chest x-ray and electrocardiogram were within normal limits.

Six blood cultures taken sequentially at 10 to 30-minute intervals after admission, were reported initially as growing an "alpha-hemolytic" streptococcus. Subsequent characterizations of the isolate, as outlined below, showed it to be Aerococcus viridans. The murmur never changed during hospitalization and an echocardiogram of the mitral valve revealed a billowing leaflet (Fig. 1A).

Back pain persisted and one week after admission tomography of the lumbar spine was performed revealing several destructive lesions on the bone cortices of both sides of the L4-L5 disc space (Fig 1B). Culture of Craig needle biopsy material from the disc space also revealed Aerococcus viridans.

Intravenous penicillin (18 million units daily) was administered for five weeks followed by two weeks of oral penicillin in dosages sufficient to provide blood levels of 2.5 units/ml. Temperature returned to normal five days following antibiotic therapy. Back pain persisted for six weeks before resolving, and radiographic improvement was documented ten weeks after initiation of therapy.

Bacteriology: Admission blood cultures revealed the presence of gram positive cocci, occurring singly, in pairs, tetrads, and a few short chains (Fig 2) that upon subculture to blood agar



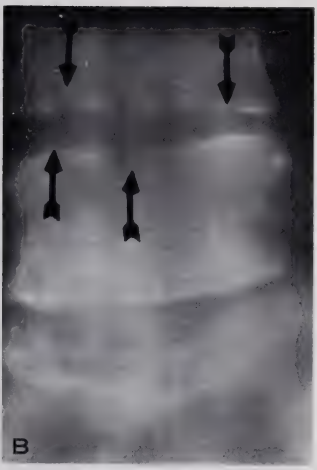


FIG. 1A. Echocardiogram of the mitral valve showing increased valvular excursion, multiple systolic echoes and marked prolapse late in systole.

FIG. 1B. Tomogram of the lumbar spine. Numerous lytic lesions are present at the L_4-L_5 interspace.

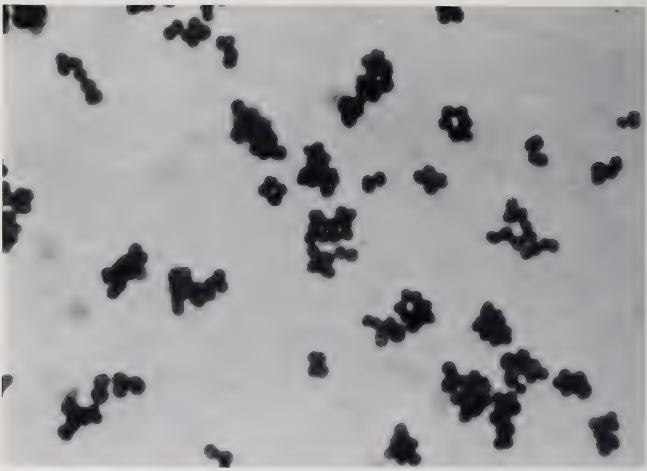


FIG. 2. Microscopic morphology of Aerococcus viridans showing tendency toward tetrad formation. (original magnification 1000x).

produced, after 24 hr incubation, small colonies surrounded by a zone of greenish discoloration of the agar medium. Based upon the Gram reaction, microscopic morphology, and colonial characteristics, the organism was initially identified as an alpha-hemolytic streptococcus of the viridans group. It was not until the subsequent isolate from the Craig needle biopsy site was noted to produce a marked greening after 48 hr incubation on trypticase soy agar containing 5% sheep blood that the possibility arose that the original as well as the present isolate were indeed aerococci. On the basis of morphologic, cultural and biochemical criteria (including esculin hydrolysis, observed as a balckening of the bile esculin medium) and on the basis of growth in differential media (such as Streptococcus fecalis (SF) broth and 6.5% NaCl agar), the isolates were identified as Aerococcus viridans (Table I). This was confirmed by Martin Weisburd of the New York City Department of Health, and through the courtesy of Dr. Richard Facklam of the Center for Disease Control. The absence of the streptococcal group D antigen was also confirmed by these investigators.

Susceptibility of both isolates, as determined by a modified two tube broth dilution method (4), showed them to be sensitive to cephalothin 7.5μg/ml, chloramphenicol 7.5μg/ml, erythromycin 1μg/ml, lincomycin 2μg/ml, penicillin 1 unit/ml, tetracycline 2μg/ml, vancomycin 5μg/ml, but resistant to sulfonamide 250μg/ml. The minimal inhibitory concentration (MIC) of penicillin for both isolates was determined to be 0.1 unit/ml.

Discussion

In most diagnostic microbiology laboratories, gram-positive cocci isolated from blood cultures which produce a greenish discloration on blood agar substrates are usually characterized as one of several streptococcal species. Unless particular attention is paid to microscopic morphology, a member of

TABLE I

Biochemical characteristics of Aerococcus viridans blood and bone isolate

Test	Result
Catalase	0
Esculin hydrolysis	+
Arginine dihydrolase	0
Hippurate hydrolysis	0
Growth on differential media:	
Endo agar	0
SF broth	+
6.5% NaCl	+
40% bile	+
Fermentation:	
Pyruvate	0
Trehalose	+
Lactose	+
Sucrose	+
Arabanose	0
Glucose	+
Penicillin MIC	0.1 units/ml
Group D Rx	0

the genus Aerococcus, which has a strong tendency toward tetrad formation, might readily be misidentified as a Streptococcus. The initial suspicion that a particular isolate might be an Aerococcus should be considered upon observing colonies surrounded by an intense zone of greenish discoloration, particularly when incubated for 48 hours.

Analogous to group D Streptococci, our Aerococcus isolates were capable of initiating growth in media containing 6.5% NaCl, Streptococcus fecalis (SF) broth, and in the presence of 40% bile as contained in bile-esculin medium. These characteristics further highlight the similarity of Aerococcus to Group D and other streptococci. With regard to the enterococci, S. fecalis and S. fecium, this distinction is of obvious importance clinically in sparing the patient therapy with an aminoglycoside antibiotic.

Late systolic murmurs, as seen in our patient, can result from the mitral valve by several mechanisms including Barlow's syndrome, Read's syndrome and primary chordal dysplasia (5–8), any of which can produce prolapse of the mitral valve during systole (7) as documented by echocardiography. Similar auscultatory findings can result from papillary muscle dysfunction secondary to ischemic heart disease (9), and while there is presently some disagreement as to the echocardiographic findings, the recent review by Teicholz found no prolapse in this disorder (10). From any cause, the altered flow patterns and regurgitant jets resulting from mitral insufficiency in these patients provide well-described sites for development of bacterial endocarditis (6, 11, 12).

Backache is a known associate of bacterial endocarditis. A review of 16 cases by Holler and Pecong (3) revealed 7 with backache as the chief complaint. Although satellite foci of osteomyelitis have not, to our knowledge, been described, vigorous use of newer radiographic tools, such as tomography, may increase the yield. The therapeutic significance of vertebral osteomyelitis as observed in this case, however, would ordinarily be minimal in a patient already receiving 4 to 6 weeks of parenteral antibiotics for bacterial endocarditis, a regimen usually sufficient to cure both lesions. While currently there is no evidence to suggest that Aerococcus is either more or less virulent than other microorganisms, increased awareness of this genus should lead to an appreciation of its role in human infections.

Acknowledgment

We are deeply indebted to Dr. Richard Bader, Dr. Edward J. Bottone and Dr. Louis Teicholz for their advice in the preparation of this manuscript.

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Reflections on the Definition and Biologic Behavior of Some Dermatologic Diseases

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For several years I have been troubled by the presence of ambiguities and misconceptions regarding a number of dermatologic diseases, especially in the realm of neoplasia. There is an obvious urgent need for sharper definitions and a better understanding of biologic concepts in dermatologic oncology. It is my intent to try to clarify and correct some of these ideas. In so doing, I fully realize not only that a number of my viewpoints may not be shared by many, but that they may also represent the antithesis of the opinions of experts in this field. However, if I shall have at least provoked and stimulated discussions which would eventually lead to a unified clear concept of any of these diseases, then the purpose of this paper will have been fulfilled.

The disorders I will discuss can be divided into four categories: dermatologic malignancies (intrinsic and extrinsic, Table I), premalignancies (Table II), potential malignancies (Table III), and pseudomalignancies (Table IV). I shall discuss certain aspects of only those diseases in each category where I feel a problem exists. The tables are quite comprehensive, as I have included many conditions, for the sake of completeness, about which there is no difference of opinion as to the biologic nature or classification among these tables. I am aware that a number of other conditions could probably be added to these categories or may, with time and future study, be eliminated or transferred to a different category. I have also permitted myself to include among the tables those lesions of the orificial mucous membrane which fall within the domain of the dermatologist.

Before entering into any discussion on this matter, a definition of terms is essential in order to understand why I have placed some diseases in a category which a priori does not seem to be appropriate. This is particularly true of some of the diseases listed under Dermatologic Malignancies (Table I). Malignancies, cutaneous or arising elsewhere, are neoplasms manifesting malignant cells histologically which may or may not present evidence of local invasion, metastases or both. Premalignancies are benign lesions showing malignant change of greater frequency than such malignancies would develop in apparently normal tissue. Potential malignancies are benign lesions in which malignant change has been shown to develop rather uncommonly or rarely. Admittedly, the separation of these diseases into two tables (Table II and III) is not based on precise statistical analysis and some of those conditions listed in Table III perhaps should be placed in Table II. Neverthe-

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TABLE I

Dermatologic Malignancies

Intrinsic

- Ectodermal Origin
  - Epithelial Tissue
    - Basal cell carcinoma
    - Carcinoma in situ
      - Intraepidermal Carcinoma
        - Actinic Keratosis
        - Arsenical Keratosis (with cellular atypism)
        - Bowen's disease
        - Erythroplasia of Queyrat
      - Intraepithelial Carcinoma
        - Leukoplakia
    - Epithelioma cuniculatum
    - Keratocarcinoma (Keratoacanthoma)
    - Paget's disease
      - Breast
      - Extramammary (apocrine type)
    - Sebaceous gland carcinoma
      - Apocrine gland
      - Eccrine gland
      - Malignant cylindroma
  - Neural Tissue
    - Malignant blue nevus
    - Malignant granular cell schwannoma
    - Malignant melanoma
      - Lentigo maligna (in situ)
    - Neurogenic sarcoma
      - Malignant schwannoma
      - Nerve sheath fibrosarcoma

- Mesodermal Origin
  - Connective Tissue
    - Epithelioid sarcoma
    - Fibrosarcoma
      - Dermatofibrosarcoma protuberans
      - Subcutaneous fibrosarcoma
    - Liposarcoma
  - Lymphoreticular Tissue
    - Lymphoma cutis
      - Mycosis fungoides
      - Sézary syndrome
    - Malignant fibrous histiocytoma
    - Malignant histiocytoma
  - Muscle Tissue
    - Leiomyosarcoma
    - Malignant hemangiopericytoma
    - Rhabdomyosarcoma
  - Vascular Tissue
    - Angiosarcoma
    - Kaposi's sarcoma
    - Lymphangiosarcoma
    - Malignant Hemangiopericytoma

Extrinsic

- Endodermal Origin
  - Epithelial Tissue
    - Extramammary Paget's disease (cloacogenic type)

less, it is important to recognize these different categories, since they have different prognostic and therapeutic implications. Pseudomalignancies are benign lesions which show histologic features of malignancy but always display a benign biologic behavior clinically.

Furthermore, in Table I (Dermatologic Malignancies) and Table IV (Dermatologic Pseudomalignancies), the diseases have been classified in terms of the primary germ layer origin (ectodermal, mesodermal or endodermal) and the type of tissue involved under each germ layer (epithelial and neural tissues of ectodermal origin, lymphoreticular, vascular, connective and muscle tissues of mesodermal origin and epithelial tissue of endodermal origin in the rare cloacogenic type of extramammary Paget's disease). For completeness, I have subdivided the Dermatologic Malignancies in Table I into intrinsic and extrinsic malignancies. By intrinsic is meant those taking their origin in the skin or orificial mucous membrane. Extrinsic malignancies may originate in any of the primary germ layers and from various tissues, but arise in organs other than the skin or orificial mucous membrane. They may be either metastatic or may represent direct extension from underlying malignancies.

Dermatologic Malignancies

I have included several diseases considered by some authorities as representing premalignant lesions (1) under the classification of carcinoma in situ, since they actually represent varying manifestations of preinvasive malignancies. I visualize leukoplakia as the clinical expression of an intraepithelial carcinoma (i.e., arising in mucous membrane) and erythroplasia of Queyrat, Bowen's disease, actinic keratosis, arsenical keratosis (with cellular atypism)

TABLE II

Dermatologic Premalignancies

- Arsenical keratosis (without cellular atypism)
- Chronic radiodermatitis
- Dyskeratosis congenita
- Epidermodysplasia verruciformis
- Giant pigmented nevus
- Nevus sebaceus of Jadassohn
- Pachyonychia congenita
- Parapsoriasis
  - En plaques
  - Variegata
- Poikiloderma
  - Atrophicans vasculare
  - Congenitale
- Syringocystadenoma papilliferum
- Xeroderma pigmentosum

TABLE III

Dermatologic Potential Malignancies

- Cellular blue nevus
- Chronic ulcer of varied etiology
- Cylindroma
- Giant condylomata acuminata of Buschke and Lowenstein
- Hidrotic ectodermal dysplasia
- Keratinous cyst
- Lichen planus
- Lichen sclerosus et atrophicus
- Oral florid papillomatosis
- Pigmented cellular nevus
- Psoriasis
- Seborrheic keratosis
- Scars
  - Chronic discoid lupus erythematosus
  - Deep fungal infections
  - Hidradenitis suppurativa
  - Lupus vulgaris
  - Polydysplastic epidermolysis bullosa
  - Smallpox vaccination site
  - Thermal burns
- Tertiary syphilis
- Verrucous epithelial nevus
- Von Recklinghausen's disease

TABLE IV

Dermatologic Pseudomalignancies

- Ectodermal Origin
  - Epithelial Tissue
    - Irritated seborrheic keratosis
    - Necrotizing sialometaplasia
    - Podophyllin therapy
    - Pseudocarcinomatous hyperplasia
      - Arthropod bite
      - Bordering chronic ulcer of varied etiology
      - Deep fungal infection
      - Granular cell schwannoma
      - Halogen eruption
      - Lupus vulgaris
      - Prurigo nodularis
      - Pseudorecidive
      - Pyoderma gangrenosum
      - Tuberculosis verrucosa cutis
    - Sebaceous duct adenoma (syringoma)
    - Trichoepithelioma
  - Neural Tissue
    - Ancient schwannoma
    - Cellular blue nevus
    - Cellular neurofibroma
    - Cellular schwannoma
    - Juvenile melanoma (spindle and epithelioid cell nevus)
    - Plexiform neurofibroma
    - Recurrent cellular nevus
- Mesodermal Origin
  - Connective Tissue
    - Aponeurotic fibroma
    - Atypical fibroxanthoma
    - Cellular dermatofibroma
    - Fasciitis ossificans
    - Infantile digital fibromatosis
    - Irradiation fibromatosis
    - Irritated dermatofibroma
    - Nodular fasciitis
    - Palmar and plantar fibromatoses
    - Pseudosarcomatous myxoma
  - Lymphoreticular Tissue
    - Actinic reticuloid
    - Arthropod bite
    - Exfoliative dermatitis
    - Hydantoin drug eruption
    - Lichen aureus
    - Lymphocytic infiltration of the skin
    - Lymphocytoma cutis
    - Lymphomatoid granulomatosis
    - Lymphomatoid papulosis
    - Mucha-Habermann's disease
    - Smallpox vaccination site
  - Vascular Tissue
    - Angiolymphoid hyperplasia
    - Capillary hemangioma of infancy
    - Irritated capillary hemangioma
    - Irritated granuloma pyogenicum
    - Papular angioplasia
    - Systemic angioendotheliomatosis proliferans

and lentigo maligna as the clinical forms of an intraepidermal carcinoma (i.e., arising in skin). I believe that all of these lesions are in situ, preinvasive malignancies because not only do they manifest the cytologic features of malignancy, but also because, given enough time, each will show histologic evidence of invasion beyond the basement membrane zone.

In agreement with Graham and Helwig (2), I believe that erythroplasia of Queyrat is a distinct clinicopathologic entity limited to the penis. Contrary to what is generally recognized, the penis is covered by skin and not mucosa. The rare case of urethral meatus involvement represents extension of this in situ malignancy from the penile skin of the glans to the mucosa of the urethra.

Although many authorities feel that a diagnosis of a malignancy cannot be made in the absence of histologic evidence beyond the basement membrane zone, I believe this to be a misconception. For the benefit of the patient, it is extremely important for the diagnosis of a malignancy to be made as early as possible, since immediate therapy for an early lesion makes for a better prognosis. In addition to the various forms of in situ malignancies already cited, I also feel that a pagetoid (superficial spreading type) malignant melanoma represents a good example of a cutaneous malignancy which oftentimes can be diagnosed before invasion of the basement membrane zone becomes manifest.

In my mind, the keratoacanthoma (so-called) is actually a special form of squamous cell carcinoma and it should be treated as a malignancy. Therefore, I have proposed that it be called a keratocarcinoma (3). Clinically, it usually manifests itself as a rapidly growing invasive tumor of relatively large dimensions and heals with a disfiguring scar. The fact of spontaneous resolution in a large percentage of cases does not prove that this is a benign lesion. The medical literature is replete with examples of well-documented malignancies of all sorts (4–6) which have undergone spontaneous regression. Furthermore, microscopically one observes an invasive neoplasm often showing the characteristic features of a squamous cell carcinoma. In fact, there are a number of cases on record of squamous cell carcinomas (7), some with metastases (8), apparently developing from lesions formerly diagnosed as keratoacanthomas. The keratocarcinoma is a distinct clinicopathologic entity showing generally a less aggressive biologic nature than the other forms of invasive squamous cell carcinoma. This diagnostic terminology (instead of keratoacanthoma or squamous cell carcinoma) will inform the clinician that he is dealing with a malignancy which shows a high rate of spontaneous resolution so that, although he will treat it as a malignancy, he may modify his therapy accordingly.

Epithelioma cuniculatum also seems to represent an unusual form of squamous cell carcinoma showing a characteristic clinicopathologic picture. The precise nosology of this neoplasm is uncertain because of the paucity of cases collected thus far. From the limited number of cases that I have been able to study, they bear a striking resemblance to the keratocarcinoma and, indeed, may possibly represent such. Because this lesion is a malignancy I believe that the appellation epithelioma is incorrect and misleading, since it indicates a benign tumor, and should not be used.

I have several comments to make regarding the basal cell carcinoma. Because there is unanimous agreement that this is a malignancy, the term basal cell epithelioma should be abandoned, since, as already stated, the word epithelioma connotes a benign growth.

Basal cell carcinomas termed as 'multicentric' and 'superficial spreading' are incorrect and misleading to the clinician. The appellation 'multicentric' implies the development of multiple basal cell carcinomas independently in a localized field of skin. As is generally accepted (9–11), every basal cell carcinoma develops from one primary focus. The term 'superficial spreading' connotes a lesion that invades only the superficial portion of the skin. I have seen many such lesions invade deeply. Therefore, in order to accurately describe such lesions, I propose that the term "creeping,' which is not new

(10), be used in place of 'multicentric' and 'superficial spreading' for this particular type.

Although the so-called fibroepithelioma of Pinkus is considered to be a premalignant lesion by some authorities (1, 9), I believe that this lesion is, in fact, a basal cell carcinoma ab initio. The histologic pattern which is considered to be peculiar for this lesion is often found in combination with a variety of other more common histologic types (Fig. 1). Since I feel that this is a malignancy, for the reason previously stated, the epithelioma portion of the appellation fibroepithelioma should be abandoned.

Is there such an entity as an in situ or intraepidermal basal cell carcinoma? I feel that such a lesion does exist. The mere fact that the basalioma cell must have originated within the confines of the basement membrane zone makes this a truism. However, such a lesion is particularly difficult to demonstrate histologically because in a basal cell carcinoma the malignant cells remain only for a relatively short period of time within the confines of the basement

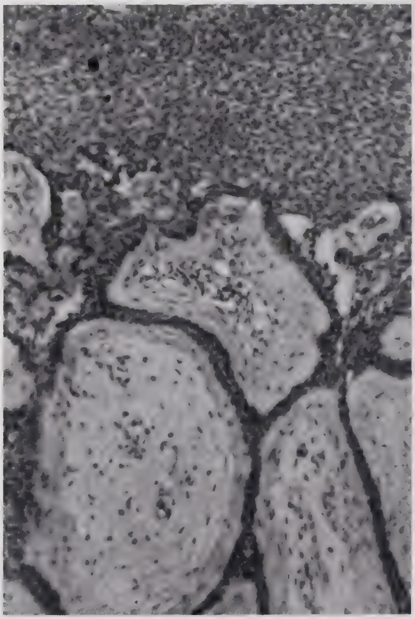

FIG. 1. Basal cell carcinoma showing combination of solid and so-called fibroepithelioma of Pinkus Pattern (H & E × 100).

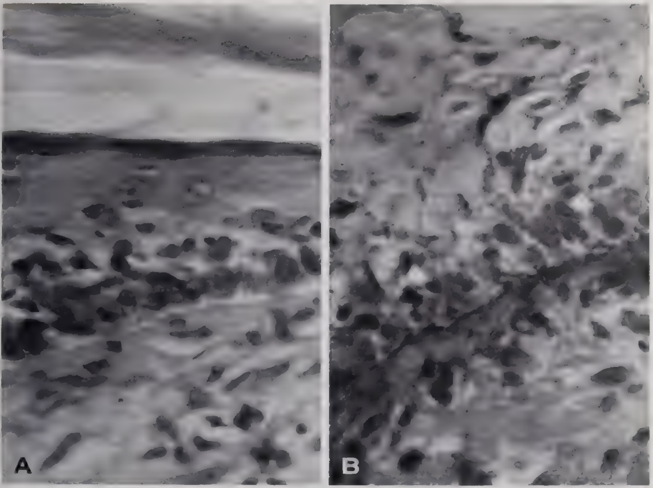

FIG. 2A. Early basal cell carcinoma showing intraepidermal malignant cells above intact basement membrane zone (H & E × 400).

FIG. 2B. PAS stain of Figure 2A emphasizing intact basement membrane zone (×400).

membrane zone. By the time the lesion is clinically manifest, invasion has already occurred in most instances. However, in very early creeping basal cell carcinomas, it is not uncommon to observe portions of or the entire lesion demonstrating in situ carcinoma (Fig. 2A). Special stains in these cases clearly show that the basement membrane zone is intact (Fig. 2B) including those cases showing retraction artifact.

Contrary to Graham and Helwig (2) and in agreement with Lever (1), I do not believe that the so-called intraepidermal epithelioma of Jadassohn represents a form of carcinoma in situ (basal or squamous cell). It is a benign lesion most often containing well-defined intraepidermal nests of uniform basaloid cells representing, in the majority of instances, a form of seborrheic keratosis. The apparently documented cases which have shown histologic and clinical evidence of aggressive biologic behavior probably represent malignant changes in seborrheic keratoses, a not uncommon occurrence (12).

As stated earlier, dermatologic malignancies may be intrinsic and extrinsic. Since I believe that Paget's disease of the breast always originates within the mammary ducts of the nipple or areolar area either within the confines of the dermis or epidermis, I consider this lesion to represent an intrinsic malignancy. In other words, Paget's disease of the breast always represents extension of a primary intraductal intracutaneous malignancy to the nonadnexal portion of the epidermis. Extension may occur along or through the ducts, via the lymphatics, via the blood stream, and from already invasive duct carcinoma within the dermis. The same line of reasoning holds for extramammary Paget's disease of apocrine duct origin. This is also true of the cloacogenic type of extramammary Paget's disease (although of endodermal origin) with the exception that the primary focus may develop from muciparous glands within mucous membrane as well as skin. Also, as has recently been reported (13), Paget's disease may occur as a result of direct extension from a metastatic breast carcinoma to the skin in an extramammary location.

In those instances in which the primary site had not been identified, I believe that either it had been overlooked because of its extremely small size or it was inconspicuous or obliterated because of origin within the intraepidermal portion of an apocrine or mammary duct or origin within the intraepithelial portion of a muciparous gland. Although the alternative view of malignant mucinous metaplasia of epidermal or epithelial keratinocytes cannot be excluded with certainty, this theory seems highly unlikely. The facts that not only are the "Paget cells" morphologically identical to the tumor cells within the primary malignant neoplasm when it is identified, but also that Paget's disease always develops in regions where muciparous glands normally occur, represent further evidence against this latter theory.

Dermatologic Premalignancies

As you will note in Table II, I have included parapsoriasis and poikiloderma in this category. In all likelihood, those cases which finally eventuate into frank lymphoma probably represent malignancies ab initio which could not be appreciated as such because so far our relatively unsophisticated technique of light microscopy is apparently not refined enough to detect the early lymphomatous cellular components.

Dermatologic Potential Malignancies

Contrary to the opinions of some experts, malignant changes do occur in seborrheic keratoses and are not uncommon. I have had the opportunity of observing malignant transformation of seborrheic keratoses into either basal cell carcinomas or in situ carcinomas (12, 14). In all likelihood, there are some cases previously diagnosed as Bowen's disease and squamous cell carcinoma which represent malignant changes that have developed within seborrheic keratoses. As previously indicated, this probably also holds true for those cases interpreted as in situ, invasive and metastatic carcinoma developing in lesions diagnosed as intraepidermal epithelioma of Jadassohn (2). I have also observed the development of so-called keratoacanthomas in seborrheic keratoses (15). For reasons previously stated, this should be considered yet another form of malignant change (keratocarcinoma) occurring in seborrheic keratoses.

In spite of the often quoted statement of malignant change in a lesion of tertiary syphilis, authentic documentation in the medical literature seems rare (16).

Dermatologic Pseudomalignancies

I have listed in Table IV a number of conditions which, in the light of both personal experience and the experience of others, have presented as cutaneous pseudomalignancies. An irritated seborrheic keratosis of relatively prolonged duration may be extremely difficult to differentiate from a basal cell carcinoma. In these instances, I have found it helpful to study the nuclear chromatin pattern (14). In basal cell carcinomas the chromatin particles are coarse and crowded together, presenting a stippled pattern, while in seborrheic keratoses having a prominent basaloid cell component the nuclei present fine, evenly spaced, inconspicuous particles forming a lacy network. The presence of conspicuous melanin pigment within the lesion is an additional aid. In seborrheic keratoses, the melanin granules are fine and golden and form a compact peri-nuclear cap. In basal cell carcinomas, the melanin granules are coarse, dark brown, anisocytotic, and randomly distributed.

In addition to the other pseudomalignancies listed under vascular tissue, it is not generally appreciated that an irritated granuloma pyogenicum or capillary hemangioma may occasionally present histologic features virtually impossible to differentiate from Kaposi's sarcoma.

Summary

Dermatologic disorders may be categorized into malignancies, premalignancies, potential malignancies, and pseudomalignancies. Actinic keratosis, arsenical keratosis (with cellular atypism), Bowen's disease, erythroplasia of Queyrat, leukoplakia and lentigo maligna are all in situ preinvasive malignancies. The keratocarcinoma (so-called keratoacanthoma) is a malignant lesion. Epithelium cuniculatum, which may be a keratocarcinoma, represents a form of squamous cell carcinoma and the appellation epithelioma which indicates a benign process should not be used in the name of this lesion. For the same reason, the term basal cell epithelioma should be abandoned and replaced by the term basal cell carcinoma. The so-called fibroepithelioma of Pinkus represents a basal cell carcinoma showing a relatively uncommon histologic pattern. For the reason stated above, the epithelioma portion of the appellation fibroepithelioma should therefore be abandoned. The term creeping should replace the incorrect terms multicentric and superficial spreading for this particular type of basal cell carcinoma. An in situ basal cell carcinoma does exist. The so-called intraepidermal epithelioma of Jadassohn is a benign lesion and is not a form of carcinoma in situ. Paget's disease always represents extension to the nonadnexal portion of the epidermis from a neighboring carcinoma, be it mammary or extramammary in location.

Although I have placed parapsoriasis and poikiloderma into the category of premalignancies, those cases which finally eventuate into frank lymphoma probably represent indiagnosable malignancies ab initio. Basal cell carcinomas, in situ carcinomas and keratocarcinomas (so-called keratoacanthomas) may develop within seborrheic keratoses. Malignant changes in lesions of tertiary syphilis are rare. Irritated and pigmented seborrheic keratoses may be distinguished from basal cell carcinomas by the morphology of the nuclear chromatin and melanin granules. An irritated granuloma pyogenicum or capillary hemangioma may be difficult to differentiate from Kaposi's sarcoma histologically.

Acknowledgment

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Electrocoagulation of Rectal Cancer: Updated

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Electric energy was first applied transendoscopically in 1910 by Edwin Beer of The Mount Sinai Hospital. Electrocoagulation (also known as 'fulguration' or 'surgical diathermy' or 'electrothermia'), as a definitive or palliative treatment for adenocarcinoma of the rectum, has been practiced since 1935 largely through the persistent efforts of A. A. Strauss of the Michael Reese Hospital in Chicago. My interest in this modality has continued from that time on to date in spite of discouraging results in many instances.

In 1968 there appeared in the "Medical News" section of the *Journal of The American Medical Association* (206:1706, Nov. 18, 1968) an article regarding electrocoagulation in the treatment of cancer of the rectum with the subtitle "Fulguration Results 'at Least as Good, if not Better,' than Surgery—New York Surgeon." I have since been asked by many internists and surgeons whether the foregoing parallels my experience, or more specifically, whether the Miles abdominoperineal resection of the rectum is dead. Stearns (1) felt that "It is distressing that our nationally respected elder statesmen in surgery have been unwilling to urge caution in this matter." It is known that *problem* patients are referred for electrocoagulation by some "elder" surgeons who apparently desire "to pass the buck."

In the past I have emphasized that cancer of the rectum actually represents three distinct disease processes, each corresponding to one of the grades of Dukes' classification. Dukes' A cancer is believed to yield a "cure" rate of almost 100 percent after any therapeutic procedure as measured in terms of 5-year survival, whereas Dukes' C carcinoma has a survival rate of less than 30 percent. By lumping all grades together, a 5-year survival statistic is arrived at which is neither fair nor accurate. Each component of any cancer classification should be considered in itself.

As a result of a rich and collective experience (2–5) throughout the years in several medical centers and private practive, I have formulated the following indications for the employment of electrothermia:

1. Dukes' A and Astler-Coller's B1 carcinoma (small, superficial lesions, especially when located on the posterior or lateral rectal walls);
2. Patients who represent poor surgical risks: e.g., advanced senility and/or concomitant serious systemic disorders;
3. Blind or senescent institutionalized patients who are unable to care for themselves or cannot procure adequate care;
4. Bleeding from inoperable lesions or metastases but with short life expectancy;

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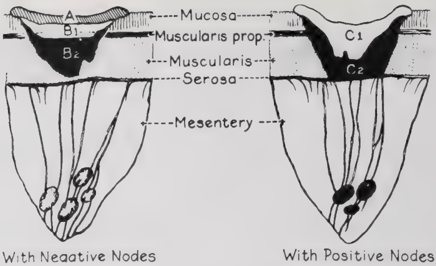

FIG. 1. Astler-Coller modification of Dukes' classification: A, Lesion is confined to mucosa; B1, Lesion extends into muscularis mucosae, but not through it; B2, Lesion extends through muscularis mucosae (From dePeyster and Gilchrist: Surg Clinics N Amer 35:1295, 1955).

5. Recurrence at the suture line following resection and anastomotic procedures; and
6. Refusal of operation.

The crux of this problem is invasion of the bowel wall. I have found electrocoagulation ideal in over 100 cases for the treatment of superficial nonnucleated polypoid lesions which fall into the classification of Dukes' A or the Astler-Coller B1 classification. The latter is a modification of Dukes' method of classification by microscopic zoning of the depth of penetration of the neoplasm in the intestinal wall. The B1 behaves clinically or biologically like Dukes' A. A precisely correct diagnosis, however, is difficult to make on even multiple fractional biopsies. Total biopsy is the answer. However, total biopsy is an almost impossible task primarily because of the flatness of the lesions. The most accepted criterion of invasion is involvement of the muscularis mucosae; this concept is justified for purposes of therapeutic orientation and for teaching considerations. Involvement of the submucosa or the lamina propria is not considered invasive cancer by a preponderance of surgical pathologists.

The embolic route of dissemination of malignancy should not be overlooked. It is known that cancer cells may fortuitously enter the lymphatic or blood vessels and thus cause regional or distant metastases without direct invasion of the muscularis mucosae or the intestinal wall. This phenomenon apparently occurs very rarely.

In eleven adenocarcinomas (with doubtful invasion) the Miles operation was preferred to coagulation as the safer procedure. In each of these cases examination of the operative specimen showed invasion of only the submucosa; in none were the regional lymph nodes involved. In retrospect these eleven patients could have been successfully treated by electrocoagulation. Incidentally, one postoperative death might have been prevented.

As already intimated, the pathologist plays a critical role. Much depends on the training, experience, judgment, and philosophy of the pathologist. It is known that in questionable instances a diagnosis of cancer is sometimes rendered by some pathologists for fear of missing one. During 1947 and 1948 I encountered, in one hospital, 22 patients with rectal neoplasms that had been diagnosed, on the basis of fractional biopsy, as "adenocarcinoma" (without reference to invasion) by the staff pathologist. These lesions were successfully

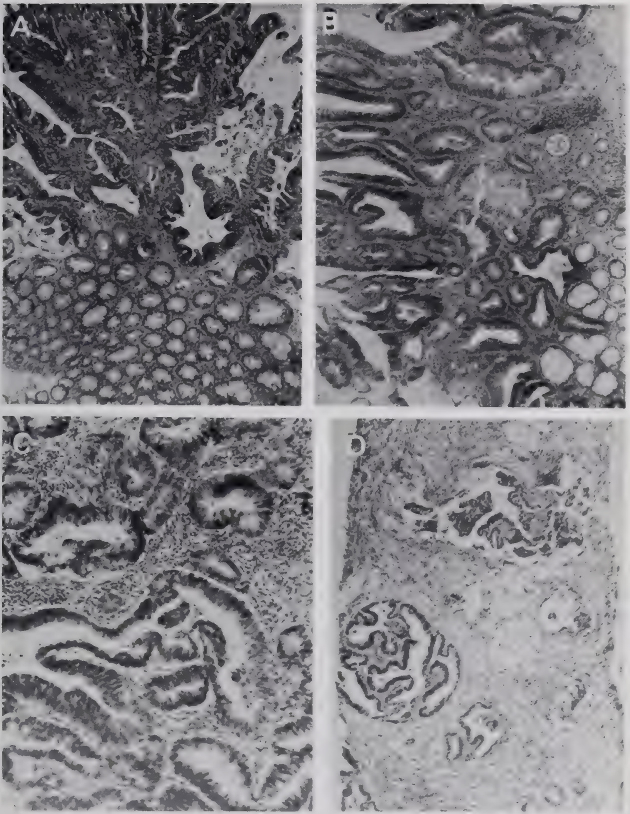

FIG. 2. A, Superficial or mucosal noninvasive adenocarcinoma; B, More advanced superficial lesion but without involvement of the muscularis mucosae; C, Minimal invasion of lesion (B1); D, Adenocarcinoma with infiltration of muscularis mucosae (From Rosenthal and Turell: JAMA 167:1602, 1958).

treated by electrocoagulation and followed for over three years. In anticipation of publication of this small series of "cancer cures" I submitted the slides to the late Drs. Klemperer and Otani who, much to my astonishment, diagnosed all of these 22 lesions benign. This, of course, was in keeping with the therapeutic success.

Good results (disappearance of the lesion) have been noted following electrocoagulation of 28 patients with infiltrating adenocarcinomas (Dukes' B & C variety) in a follow-up of over 5 to 15 months. This experience was decidedly in contrast to the poor results obtained in some 150 patients with similar lesions diagnosed by the same pathologists. Partly for this reason I electrocoagulate invasive lesions in patients who for valid reasons are denied surgical resection. Though this small group of patients constitutes an insignificant overall percentage, the results are nevertheless major therapeutic successes in the lives of each of the involved individuals. Since good therapeutic results following electrothermia are exceptional in patients with ulcerating, invasive cancer (Dukes' B & C), I continue to advocate the Miles procedure as the first choice.

Some patients with infiltrating cancer were made clinically worse following electrocoagulation as evidenced by an increase of tenesmus or bleeding or both. Following treatment these individuals could not tolerate instrumentation, e.g., observation endoscopy, without regional anesthesia. Other patients with similar lesions however, have obtained considerable palliation.

Variable degrees of palliation were obtained in more than a score of patients with recurrence of cancer at the suture line following anastomotic procedures —anterior resection. Bleeding was invariably controlled as were the obstructive symptoms caused by stenosing of the lumen. The contracting lumen was infrequently widened by coagulation.

For some time it was believed that the good results ("cures") following physical destruction and necrosis of the tumor tissue were brought about merely by the application of electrical energy. However, Strauss, Appel, and Saphir (6) have shown experimentally that necrotic neoplasms serve as antigens eliciting a specific antibody response that retards the coagulated lesions. When partial electrocoagulation was affected, necrosis, regression, and even disappearance of the original neoplasm occurred. In short, the "cures" ascribed to electrothermia appeared to be the result of an induced immunologic response. By treating a tumor in stages, bionecrosis is believed to develop between the area of necrosis and viable tumor which, in turn, produces a systemic immunologic reaction similar to that produced by cryotherapy. This tenet, however, is questioned by Cole (7) and others.

In sum, electrocoagulation is an *old* procedure and is recommended for the treatment of adenocarcinoma of the rectum (1) in selected, especially poor risk patients; (2) on a trial basis for patients who refuse operation; and (3) unequivocally for the patients in whom a diagnosis of Dukes' A or Astler-Coller B1 cancer can be established with certainty. In spite of the infrequent apparent therapeutic success in instances other than Dukes' A lesions, electrothermia should not be regarded as definitive therapy for invasive adenocarcinoma of the rectum.

The technique of electrosurgical management is relatively simple. Patience and dexterity with the use of armamentarium, as described elsewhere (3), is of prime importance.

Incidentally, the value of freezing or the cryotechnique in the treatment of benign and malignant intestinal neoplasms is in the process of personal investigation.

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Bronchial Asthma with Acute Respiratory Failure

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Abstract

This case report describes in detail the fatal clinical course, laboratory and roentgenographic studies, treatment, and postmortem findings in a 26-month-old male child with severe bronchial asthma and terminal acute respiratory insufficiency. The deleterious effects of long-term steroid therapy are considered as a possible mechanism to explain the patient's rapid demise.

There is at the present time some controversy over whether or not there is an increasing mortality rate in childhood asthma. The trend toward increasing mortality was reported first from England and Wales (1–5) and later from Australia (6, 7). However, studies from Canada (8) and United States (9) have not confirmed this trend, but rather seem to indicate that the converse may be true.

Proponents of the theory of increasing mortality have implicated the increased use of bronchodilator aerosols, adrenal cortical steroids, and sedatives for the increase. However, these implications have been inferred primarily from statistical studies.

This case report describes the fatal clinical course and postmortem findings in a 26-month-old male with bronchial asthma complicated by acute respiratory failure. The prior use of adrenal cortical steroids seemed to unfavorably influence his clinical course.

Case Report

The patient, a 26-month-old white male, was admitted to St. Vincent's Hospital for detailed study and treatment of recurrent bronchial asthma.

The child developed the first episode of bronchospasm at one month of age and then continued to have recurrent, non-seasonal asthmatic attacks throughout the next twenty-five months of life. He had seven prior hospitalizations for status asthmaticus precipitated by broncho-pulmonary infections before his admission to St. Vincent's Hospital. As an outpatient he was refractory to the usual therapeutic modalities used for allergic asthma. His outpatient treatment included sympathomimetic amines, antihistamines, antibiotics, gamma globulin, adrenal cortical steroids, elimination diets, and hyposensitization therapy. The steroids were started on the patient at two months of age and continued until approximately two months before admission.

Physical Examination: Revealed a 14 Kg white male with mild Cushingoid features. He was in no acute distress on admission and the vital signs were normal. Expiratory wheezes were noted bilaterally over all lung fields.

Laboratory Examinations: Hemoglobin, hematocrit, and white blood cell count were within normal limits, however the differential white blood count showed 20% eosinophils. An absolute eosinophil count was 1782 per cu mm. Erythrocyte sedimentation rate, nose and throat cultures,

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immunoglobulins, serum electrolytes, a sweat test, alpha, antitrypsin, and urinalysis were either negative or within normal limits.

Roentgenographic Examinations: A posterio-anterior and lateral roentgenogram of the chest showed heavy peribronchial markings at both hilar areas. The lung parenchyma was normal.

Hospital Course: On admission, the child was treated with a modified hypoallergenic diet, bed rest, expectorants and oral bronchodilators. He remained comfortable until the morning of the third hospital day when he developed respiratory distress. Physical examination revealed expiratory and inspiratory wheezes bilaterally. Arterial blood studies at this time (9:00 AM) were as follows: pH 7.36; pCO_2 38 Hg; HCO_3 21 mEq per liter; pO_2 51 mm Hg and oxygen hemoglobin saturation 86%.

The child was now treated with 40% humidified oxygen, intravenous fluids, aminophylline by intravenous infusion, and erythromycin orally. He was followed closely and at 11:00 AM, he was found to have bilateral subcutaneous emphysema in the submandibular area, in the neck and over the upper chest wall. The lung findings were unchanged on physical examination. Roentgenographic study of the chest confirmed the physical findings of subcutaneous emphysema and showed no evidence of pneumothorax, pneumomediastinum, atelectasis, or pulmonary infiltrates. Arterial blood gas studies at this time (11:00 AM) were as follows: pH 7.37; pCO_2 27 mm Hg; HCO_2

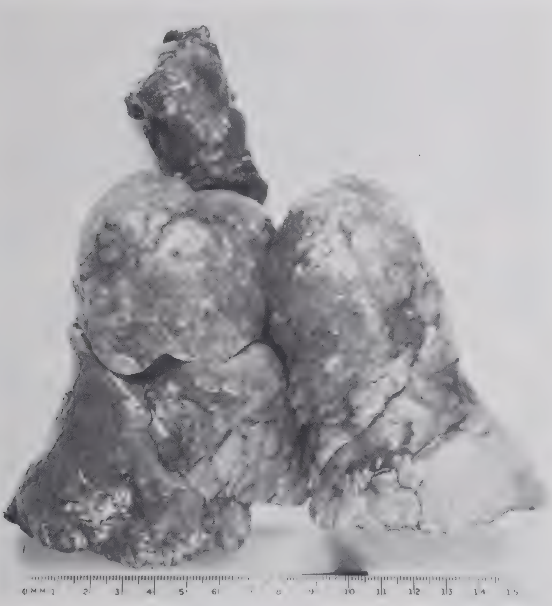

FIG. 1. The lungs exhibit extensive collapse of the left upper and most of the left lower lobes. The remainder of the left lower lobe shows emphysematous changes and bleb formation in the anterior medical basal segment. The right lung is pale, soft, voluminous, and weighs 150 grams. There is also interstitial emphysema with bleb formation in the anterior and lateral basal segments of the right lower lobe.

15 mEq per liter; pO_2 45 mm Hg and oxygen hemoglobin saturation 79%. Intravenous hydrocortisone was now added to the treatment regimen.

At 11:30 AM, the child developed apnea. Mouth-to-mouth resuscitation followed by manual ventilation with a self-inflating resuscitation bag was started immediately. The child was then intubated with a #20 endotracheal tube, and large amounts of thick tenacious brown mucus were aspirated from the trachea and bronchi. He was then started on a volume cycle respirator and given sodium bicarbonate. Immediately after adequate respiration was restored, nasogastric aspiration yielded 200 cc of coffee-ground-type material.

Serial arterial gas studies while the patient was being treated with the volume cycle respirator are listed in table one.

The child was subsequently treated with alkali, continued mechanical ventilation, hydrocortisone, antipyretics and a hypothermic mattress.

Despite the respiratory intensive care therapy, the child remained acidotic and refractory to bicarbonate therapy. At 7:00 PM, he developed dilated, fixed pupils. Thirty minutes later he developed cardiac arrest. He was resuscitated by external cardiac massage and intracardiac injection of epinephrine. Now isoproterenol was needed to maintain blood pressure. However, the patient gradually became refractory to this therapy. Examination at 11:00 PM revealed absent breath sounds and increased resonance over the left lung. Roentgenographic examination revealed a 40 percent pneumothorax of the left chest. The patient died before further therapy could be instituted (Table I).

Postmortem Examination: The necropsy was performed twelve hours after death. The significant findings were as follows:

(a) External examination showed a young male with Cushingoid features and subcutaneous emphysema of the neck and anterior chest wall;

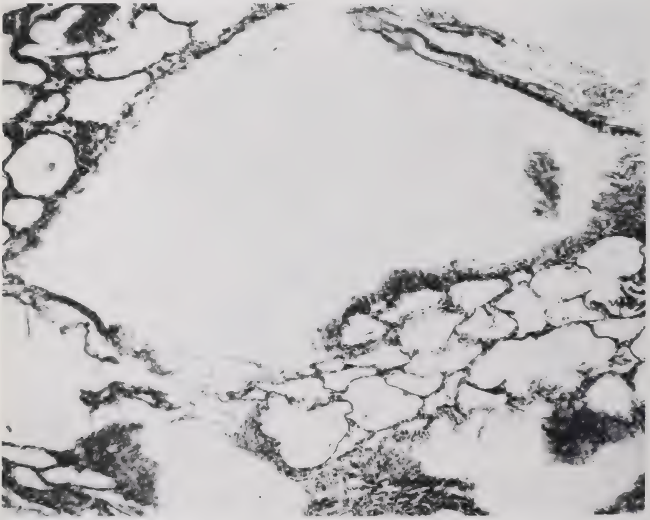

FIG. 2. There is extensive alveolar rupture with interstitial air dissection and pleural bleb formation.

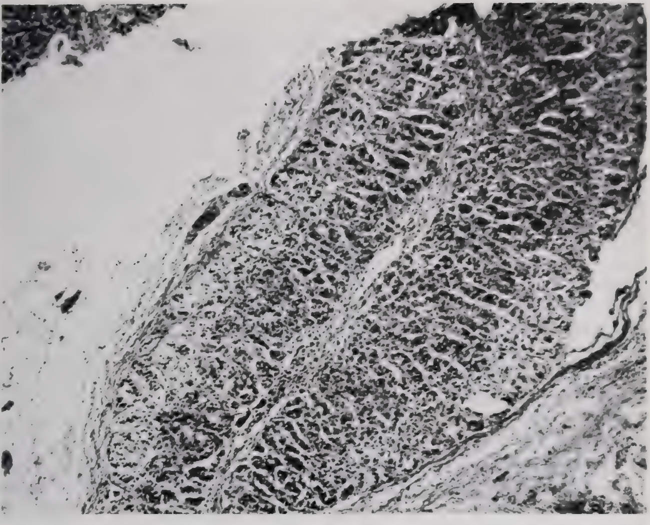

FIG. 3. The adrenal glands are atrophied to approximately 50% of normal size. On section the lipid content is depleted.

TABLE I

Course of the Patient

Time	pH	mm Hg pCO_2	mEq/l HCO_3	mm Hg pO_2	Sat.
11:30 AM	7.37	27	15	45	79%
2:30 PM	7.04	69	17.5	69	83%
3:00 PM	7.26	33	14	64	87%
3:55 PM	7.14	44	14	68	86%
5:10 PM	7.16	61	20.5	69	87%
6:20 PM	7.22	44	17.1	76	91%
7:00 PM	6.96	52	10.7	144	96.8%
8:00 PM	7.06	64	16.8	180	98.5%
8:20 PM	7.13	40	13.2	152	98.1%
8:50 PM	7.12	48	14.7	54	76.3%
10:00 PM	7.10	45	15.2	200	99%

(b) The chest cavity was opened under water. Air under pressure was found in the left hemithorax. There was also approximately ten cc of air in the pericardial sac;

(c) The right lung was pale, soft, voluminous and weighed 150 grams. There was marked interstitial emphysema with bleb formation in the anterior and lateral basal segments of the lower lobe. The left lung showed extensive collapse of the left upper and most of the left lower lobe. The remainder of the left lower lobe showed emphysematous change and bleb formation. The bronchi of both lungs were hyperemic and filled with viscid mucus plugs. Microscopically there was thickening and hyalinization of the basement membrane in the medium and larger sized bronchi. The bronchial lumen contained copious amounts of mucus admixed with desquamated epithelial cells, eosinophils, round cells, and polymorphonuclear leukocytes and Curschmann's spirals. In addition, there was hypertrophy of the bronchial musculature, hyperplasia and hypersecretion of the bronchial glands, and eosinophilic infiltration of the bronchial walls. Some sections revealed alveolar rupture with interstitial air dissection and pleural bleb formation;

(d) The adrenal glands were atrophic. Each weighed three grams. On section the cortical lipid content was depleted; and

(e) There were two acute peptic erosions in the esophagus, 2.5 cm. from the cardio-esophageal junction. There was also an acute erosin at the cardio-esophageal junction along the lesser curvature. Microscopically these erosions showed necrosis with a minimal polymorphonuclear response.

Discussion

Acute bronchial asthma is a well known but infrequent cause of severe respiratory failure. However, most patients respond well to therapy, particularly if adequate ventilation is maintained and the patient is prevented from becoming severely hypoxic or hypercapnic. The patient in this case is an exception to this rule.

Tension pneumothorax and/or pneumomediastinum are known possible grave complications that may occur in the patient with status asthmaticus. The immediate cause of death in this patient was the development of a tension pneumothorax. However, when this event occurred, the patient was in extremis and other factors seemed to be of greater importance in influencing his demise.

In this child, the most important factor which seemed to influence his lack of response to therapy was the deleterious effects caused by long-term adrenal cortical steroid therapy. The diffuse and unresolved infection of the bronchial wall, the patient's inability properly to react to the stress of the repiratory failure and the stress ulcerations of the esophagus and stomach can be attributed to long-term steroid therapy.

It is also tempting to speculate on the possible contributory role of chronic steroid therapy as a cause of the anatomic changes in the pulmonary parenchyma (i.e., bleb formation with terminal pneumothorax).

It has been demonstrated (10) that some children treated with steroids have static volume-pressure curves similar to that found in incipient emphysema. Significant increases in pulmonary compliance as well as abnormal bronchial collapsibility and pressor amine resistance have also been demonstrated. The above-mentioned changes, combined with chronic active bronchial inflammation, retained tenacious mucus, secondary adrenal gland atrophy, predisposed to agonal bleb formation and rupture with tension pneumothorax.

We hope, therefore, that this case will serve as a continuing stimulus for a critical evaluation of steroid therapy in the child with asthma.

Acknowledgments

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Primary Malignant Neoplasms of the Small Bowel

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Hamburger in 1746 (1) was the first to describe a small bowel carcinoma. Since that time approximately (2200) cases of primary small intestinal malignancies have been described in world literature. Incidence ranges from 0.2 to 5% of all gastrointestinal malignancies. Variability is dependent on whether one considers lymphomas, ileocecal valve, and periampullary lesions in the study. Especially with lymphomas, it is difficult to prove primary origin. Elimination of the above three lesion locations, decreases the incidence to between 0.2% and 2%.

The major tumors found in order of frequency of occurrence are adenocarcinoma, carcinoid and leiomyosarcoma. Other extremely rare malignant masses are rhabdomyosarcoma, liposarcoma, angiosarcoma, fibrosarcoma, and neurogenic sarcomas.

Adenocarcinomas account for approximately 50% of all small bowel malignancies; then come carcinoids, approximately 35%; and leiomyosarcomas approximately 15%. The other rare sarcomas are all cumulatively under 0.5%. If we include lymphomas, these fall in incidence between the adenocarcinomas and carcinoid tumors.

Table I shows the distribution of these tumors. It can be seen that the adenocarcinomas occur in highest incidence first in the duodenum, followed closely by jejunum, and least in the ileum. Carcinoids are predominantly ileal lesions, while leiomyosarcomas also have a propensity to arise in the ileum.

On further breakdown of duodenal location, Kleinerman (2) found that 40% arise suprapapillary, 40% infrapapillary and 20% peripapillary. This study was done after excluding 219 of 268 peripapillary tumors because of other than intestinal origin. If all peripapillary masses are included, then it's incidence rises to approximately 65%.

Malignant lesions are divided evenly between males and females. Some authors such as Reyes (3), Sethi (4), and Ostermiller (5) have shown mild male predominance.

Greatest incidence with regard to age is as shown in Table II.

Clinical presentation of disease varies, but at least 90% of all patients are symptomatic by time of diagnosis (6, 7). This is invariably due to the difficulty in diagnosing these disease entities. Table III (8) lists the clinical manifestation of small bowel tumors and compares malignant with benign diseases.

Pain is usually described as intermittent cramping abdominal pain which is

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TABLE I

Distribution of Malignant Tumors of Small Intestine
2220 Cases

	Total	Duodenum	Jejunum	Ileum
Adenocarcinoma	1050	418 (40%)	399 (38%)	234 (22%)
Carcinoid	731	54 (7%)	75 (10%)	622 (83%)
Leiomyosarcoma	439	43 (10%)	160 (37%)	236 (53%)

TABLE II

Adenocarcinoma	— 7th decade followed closely by 6th
Leiomyosarcoma	— 6th decade
Carcinoid	— Variable from report to report with most tendencies in the late 6th and 7th decades.

TABLE III

*Clinical Manifestations of Small Bowel Tumors**

	Malignant Tumors	Benign Tumors
Symptomatic	30 (94%)	18 (53%)
Weight Loss	17 (53%)	6 (17%)
Pain	25 (47%)	14 (40%)
Anemia	13 (41%)	4 (40%)
Nausea or vomiting	11 (34%)	8 (24%)
GI bleeding	8 (25%)	11 (32%)
Mass	7 (22%)	1 (3%)
Obstruction	6 (19%)	7 (21%)
Diarrhea	5 (16%)	3 (9%)
Constipation	4 (13%)	2 (6%)
Jaundice	2 (6%)	0 (0%)
Total no. patients	32	34

* Data presented in this table compiled from statistics found in Silberman et al (8).

typical of partial bowel obstruction. Epigastric burning or dull aches have also been reported.

Bleeding of the gross or occult type may be present. Patient's families may have noted a progressive pallor and the patient will often complain of decreasing stamina and increased fatigue. Massive hemorrhage may be the dramatic presentation of leiomyosarcoma, and is relatively more common with this tumor than with adenocarcinoma or carcinoid.

Obstruction may also occur or even more rarely obstruction secondary to intussusception. Obstruction is more characteristic of carcinoma than sarcomas. This is because the former grow in an annular constricting fashion, whereas the latter compress the lumen only after reaching large size. Polypoid adenocarcinomas are the most likely lesion to initiate intussusception, rarely it may be due to carcinoid.

The most common physical finding is an abdominal mass. However, it must be realized that a large number of these so called masses are in fact not tumors, but 1) intussuscepted bowel, or 2) dilated proximal small bowel. Masses associated with jejunal tumors are generally located in the left quadrant, while those associated with ileal lesion are in the lower abdomen (9).

Jaundice is usually an accompaning presentation with periampullary lesions. However, extensive secondary portion duodenal tumors may also make the patient icteric.

One rare complication is perforation, present in under 3% (9) of all cases. Most are reserved to the ileum.

Length of symptomatology varied from report to report, but most symptoms began six months to 1-2 years prior to diagnosis (3, 5, 8, 9) (Table IV).

In diagnosing the disease, one common source of error was repeated in all reported studies. Due to the low incidence of these malignancies, Keats (11) in 1965 demonstrated that 23% of missed diagnoses were demonstrated on barium study.

Vuori (12) in his study of 60 small bowel malignancies, reviewed his x-ray data and showed an overall positive rate of 63%. However, 90% of these were either close to the ligament of Treitz or ileocecal value (Table V).

Approximately 50% (12) of all patients had typical X-ray findings compatible with the five major categories in Table VI. The other half had only one sign such as one distended intestinal loop. with the latter findings, these diseases are usually at the bottom of our differential diagnosis, if even considered at all.

TABLE IV

*Duration of Symptoms **

	Malignant Tumors	Benign Tumors
0–5 Days	5 (17%)	5 (28%)
5 Days–2 months	3 (10%)	1 (5%)
2 Months–6 months	4 (13%)	3 (17%)
6 Months–1 year	9 (30%)	3 (17%)
More than 1 year	9 (30%)	6 (33%)
Total no. patients	30	18

* Data presented in this table compiled from statistics found in Silberman et al (8).

TABLE V

*Results of Small Intestinal Barium Studies According to Location of Tumor**

	Positive	Negative
All Barium Studies of Small Bowel	38 (63%)	22 (37%)
Duodenum	16 (70%)	7 (30%)
Jejunum and ileum	19 (53%)	16 (47%)

* Data presented in this table compiled from statistics found in Vuori (12).

TABLE VI

*Roentgenologic Changes Caused by Malignant Tumors of Small Intestine**

Type of Change	Number of Cases
Rough or defective mucosa	26 (65%)
Harrowing of intestinal umen	24 (60%)
Peristaltic change and rigidity	22 (55%)
Eccentric movement of contrast medium	20 (50%)
Distended intestinal loops	20 (50%)
Annular, non-obstructive change	15 (38%)
Demarcated filling defect	13 (33%)
Ulcer-like lesion	7 (18%)
Dislocation of intestinal loop	3 (8%)
Fixation of intestinal loop	3 (8%)
Total no. cases	40

* Data presented in this table compiled from statistics found in Vuori (12).

Barium study can rarely determine the type of lesion present. In a few cases characteristics of different groups could be observed. The leiomyosarcoma may have a great filling defect with crater formation on the surface and dislocation of the intestinal loops. Carcinoids may manifest themselves as a small sharp line filling defect in which the mucosal pattern has remained intact.

Good (13) has identified several radiographic patterns to be kept in mind. Local filling defects in the barium study may be: 1) intraluminal, 2) mucosal or ulcerating, or 3) intramural. Intraluminal defects are most often caused by pedunculated adenomatous polyps or leiomyomas. Mucosal or ulcerating defects are most often caused by adenocarcinomas and less commonly by lymphomas. Lesions such as leiomyosarcomas or lymphomas may encroach upon the lumen while mucosal integrity remains intact. These lesion may also grow outward and produce a clinically palpable mass which may or may not correlate radiographically with displacement of adjacent small bowel loops.

During surgery it is often possible to grossly identify the type of tumor present. Adenocarcinomas are of the annular variety, leiomyosarcomas are larger with frequent cavernous formation, while the carcinoids are smaller, usually yellowish lumps under the mucosa.

The carcinomas most often spread to the para-aortal nodes, the leiomyosarcomas to the peritoneum and the carcinoids to the liver. Table VII shows the sites of distant metastases from these malignant masses. Incidence of metastases varies directly with frequency of occurrence of lesions. Thus, adenocarcinoma to para-aortal nodes, then carcinoids to the liver and leiomysarcoma to peritoneum.

The specific characteristics of adenocarcinomas make it the most common symptomatic small bowel malignant tumor. It forms approximately 90% of all duodenal malignant masses. It may present with nausea, vomiting, epigastric discomfort and occult blood in the stools. However, these can easily mimic

TABLE VII

Site of Distant Metastasis from Malignant Neoplasm of the Small Intestine[*]

Para-aortal nodes	26%
Peritoneum	19%
Liver	17%
Omentum	9%
Uterus and ovary	6%
Urine bladder	4%
Spleen	3%
Adrenals	3%
Colon	4%

[*] Data presented in this Table compiled from statistics found in Vuori (12).

simple ulceration, hiatus hernia, pancreatic or biliary disease (14). It is usually encircling, a napkin-ring-like lesion. Often it is periampullary and in many cases it cannot be distinguished grossly or microscopically from pancreatic or common bile duct lesions. Most patients have metastases by the time of recognition. Surgery offers the only chance of cure including pancreaticoduodenectomy. Operative mortality may be as high as 20–30% (12) and five-year survival in the range of 5–25% (4, 12, 15, 16). Adenocarcinomas of the jejunum and ileum are of three major types: 1) annular constricting type, with its partial or complete small bowel obstruction; 2) the irregular ulcerative type which produces bleeding, anemia, and, rarely, perforation; and 3) the polypoid type.

The most extensively studied tumors are the carcinoids. These masses have the ability to secrete serotonin, histamine, kinins, and ACTH. The typical carcinoid syndrome of diarrhea, flushing, bronchial constriction and cardiovascular problems is present in only 10% (17) of individuals with this lesion. Usually there is evidence of hepatic metastases, though liver involvement may be present without symptoms. These patients usually have 1-2 year histories of the usual complaints present with small bowel tumors. They elicit fibrotic response and obstruction may occur. Intussusception and bleeding are rare. The majority are in the ileum and are 1–2 cm in size, well circumscribed and yellow. They infiltrate outward to serosa, mesentery and adjacent lymph nodes. All should be considered malignant and 90% (18) have metastasis by the time the patient is symptomatic. Metastasis is related to tumor size. If under 1 cm, metastases are rarely observed. Multiple tumors are found in 10–30% of the cases. Research of the involved segment with associated mesenteric lymph nodes is the therapy of choice and may involve a right hemicolectomy for tumors of the terminal ileum. Carcinoid tumors grow slowly and even in the presence of liver metastases the most extensive resection possible should be undertaken. Five-year survival is in the range of 30–65% (9, 12, 18).

Approximately 60% of smooth muscle tumors of small bowel are malignant. These leiomyosarcomas are most prevalent in the ileum. They arise from the muscular coats of the bowel wall and are most frequent subserosal or exoenteric. However, they may grow toward the bowel lumen and become polypoid. They assume a round or oval shape and frequently have cavernous formation or mucosal ulceration causing its high incidence of bleeding. Leiomyosarcomas are usually slow growing and even palliative resections may yield useful results. Metastases are blood-borne, so that extensive resections to include draining lymph nodes are not warranted. Five-year survival is 20–40% (9, 12, 13).

Table VIII lists the one- and five-year survivals of all small bowel malignancies according to histologic type of tumor.

A few syndromes and diseases have shown some tendencies to cause and increase the probability of a patient to develop small bowel malignancy.

John Reid (19) in JAMA, August 1974, published a review of patients with Peutz-Jeghers Syndrome. The classical presentation is of generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. Of the cases reviewed it was demonstrated that these individuals had a 2–3% chance of developing small bowel carcinoma and that site of origin was either in a Peutz-Jegher polyp or surrounding mucosal. Highest incidence of occurrence was in the duodenum.

Evidence has also pointed to the fact that Crohn's disease may predispose to carcinoma of the small bowel. Dr. Leon Ginzburg (20) was the first to report in 1956 an adenocarcinoma of the jejunum forming in a patient with nonspecific granulomatous enteritis. Data lending to this increased incidence are 1) age of incidence (adenocarcinoma of small intestine is highest in the 7th decade, while for patients with Crohn's disease it is in the mid-5th decade), and 2) most adenocarcinomas are located in the duodenum or jejunum except in patients with Crohn's, where the highest incidence is in the terminal ileum. The overall incidence of small bowel carcinoma is 0.3 per 100,000 or 3 per 1,000,00. However, patients with Crohn's disease have a 0.3% to 0.6% or 1 in 354 to 640 chance of developing the disease. If we consider the possibility of both diseases in the same segment of bowel the correlation between the two factors greatly improves.

The relative immunity of the small bowel to the development of malignant tumors is an enigma. Many factors may come into play (9). Rapid transit time and fluid alkalinity may decrease bowel exposure time to carcinogens; or perhaps enzymes, such as certain of the hydrolases present in large quantities in the small bowel, may degrade some carcinogens. The high concentration of

TABLE VIII

Prognosis of Patients with Small Bowel Malignant Neoplasms

Tumor Classification	Survival	
	1 Year	5 Years
Carcinoma	75%	30–65%
Leiomyosarcoma	65%	20–40%
Adenocarcinoma	32%	5–25%

immunoglobulin A in the small bowel, may also help to decrease the incidence. Whatever the cause, this relatively rare state limits one's suspicion of this as a primary lesion, and by the time of diagnosis, prognosis is poor.

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Student Section

The Use of Ultrasound in the Assessment of Intrauterine Fetal Death: A Review

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Ultrasound was first used to define tissue interfaces and foreign bodies in 1949 by Howdry and Bliss (1). Eight years later diagnostic ultrasound was used to visualize the uterus and its contents in pregnancy by the Scottish obstetrician, Ian Donald (2).

The diagnosis of intrauterine fetal death (IUFD) or potentially fatal fetal malformations holds grave significance both for obstetrician and patient. Ultrasound can give information which suggests fetal death in utero within 4 to 6 hours after demise (3). Signs of fetal death may not appear on roentgenogram examination for 2 to 10 days and cannot be demonstrated in pregnancy of less than 16 weeks gestation (4). In contrast to radiologic studies in pregnancy, diagnostic ultrasound at present appears to cause no toxicity or teratogenic effects. The potential hazard of ultrasound is a subject of intense investigation (5–10).

Ultrasonic visualization of tissue structures is based on the principles of echo sounding developed in World War II. A pulsed or continuous beam of high frequency sound is transmitted into the body and reflected back to the receiver at different intervals depending on the density of the tissue. The ultrasonic echoes are electronically processed and can be displayed by cathode ray oscillography. Ultrasonic scanning for obstetric diagnosis often uses the two dimensional scan or "B-mode" (A-mode is unidimensional) which displays "sonolucent" and "sonodense" images of tissues or fluid-filled spaces in longitudinal or transverse planes through the body.

Ultrasonic patterns of IUFD vary with regard to gestational age. In early pregnancy Donald (11, 12), Hellman (13), and Leopold (3) related sonographic patterns of 1) poor gestational sac outline, 2) small-for-dates-gestational sac size, 3) presence of abnormal or absent fetal echoes, 4) uterine growth failure, 5) double gestational sac outline, and 6) low implantation position of gestational sac with blighted ovum, early abortions, and fetal death in utero. The results of a study by Hellman (13) of 141 gravida women with abnormal sonograms are shown in Table I.

Sonographic visualization of the gestational sac through an expanded urinary bladder has provided reliable information about pregnancy as early as 3–5 weeks gestation. A double gestational sac has been visualized as early as 8

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TABLE I

Results of study of 141 gravida with obstetrical difficulties having abnormal sonograms (16)

Finding*	No.	Abort	live	immature	ectopic	myoma	molar	non-preg
1—Poor GS	71	59	3	1	3	2	—	3
2—Small GS	63	59	1	1	—	—	—	2
3—Abn IUE	54	49	—	1	—	1	1	—
4—UGF	38	35	1	—	—	—	—	2
5—DGS	35	27	8	—	—	—	—	—
6—Low P	6	5	1	—	—	—	—	—

* 1. Fragment, poorly defined gestational sac outline; 2. Small-for-dates gestational sac size; 3. Abnormal or absent fetal echoes; 4. Uterine growth failure; 5. Double gestational sac outline; 6. Low implantation position of gestational sac.

weeks of pregnancy (5). Gottesfeld was able to demonstrate the trophoblastic ring as early as 4 weeks and was able to follow viability by noting collapse or disappearance of the ring in subsequent sonograms (4).

In the study by Hellman and Kobayashi (13), 71 of 140 gravidas with obstetrical problems were noted to have poorly defined, absent or fragmented gestational sac outlines on sonography. Finding a blotchy, speckled, or C-shaped fragmented trophoblastic ring or gestational sac was associated with early abortion, Fifty-nine of 71 gravidas in this group aborted. Figure 1 shows a sonogram in early pregnancy.

Fetuses 8 weeks of age and older have sonographic patterns which show regular contours without internal echoes. Forty-nine of 54 gravidas in Hellman's study (13) having sonograms showing markedly abnormal or absent fetal echoes went on to abort; one gravida had a molar pregnancy; one gravida had an intrauterine myoma. Fifty-nine of 63 gravidas in Hellman's study (13) having gestational sacs that were small-for-dates by more than 2 weeks went on to abort. Thirty-five of 38 gravidas which showed no progression in gestational sac size in serial sonograms aborted. Twenty-seven of 35 gravidas having a double sac outline aborted. It is interesting to note that the double sac suggests a double ovum and was most often found in patients using ovulatory inducers such as clomid and perganol. Of the 13 gravidas in this group who had been using ovulatory inducers, all 13 aborted.

The most ominous sign of impending abortion was the finding of a low position of implantation of the gestational sac in association with an open cervix. This finding was usually a late sign just prior to expulsion of the fetus. Five of 6 gravida women (13) with low gestational sac implantation sites aborted. The finding of a low implantation site with a closed cervix is less suggestive of impending abortion than a low implantation site with an open cervix.

One of the most reliable signs of IUFD in early pregnancy is failure to hear the fetal heart using the Doppler Ultrasound flowmeter. The principles and use of the Doppler flowmeter have been described by Johnson and others (14). Using Doppler echoes, the fetal heart sounds may be heard as early as 8 weeks gestation but most often are heard between 11–14 weeks. Some investigators

(3) suggest that if no fetal heart tones are heard by the tenth week the possibility of IUFD should be considered, while others (14) believe that the period of 10–13 weeks is "blind" for accurate ultrasonic visualization and detection of fetal viability.

Use of the Doppler ultrasound device has an advantage over ECG diagnosis of fetal heart activity. Electrocardiogram was found to be 97% accurate with regard to viability during only two gestational periods, 18–24 weeks and 32–40 weeks; it was 50% accurate during the period 24–32 weeks. In contrast the Doppler flowmeter was almost 100% accurate with regard to viability from 14

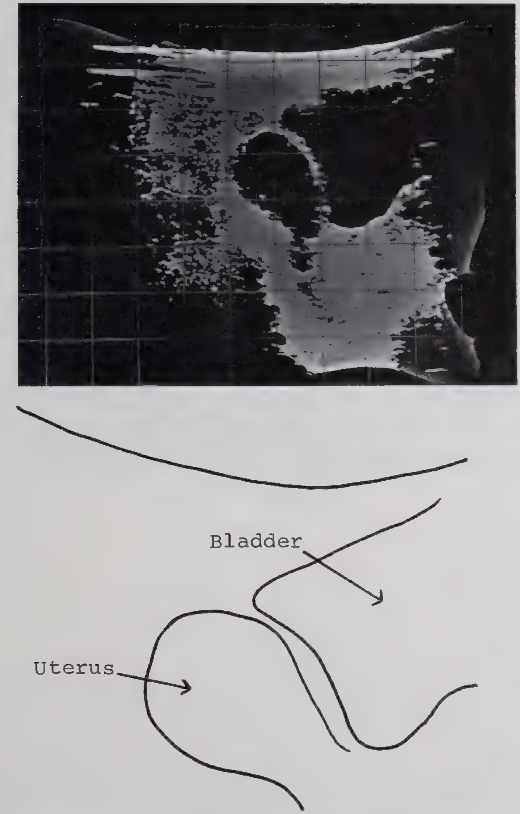

Fig. 1. Sagittal scan of bladder and uterus.

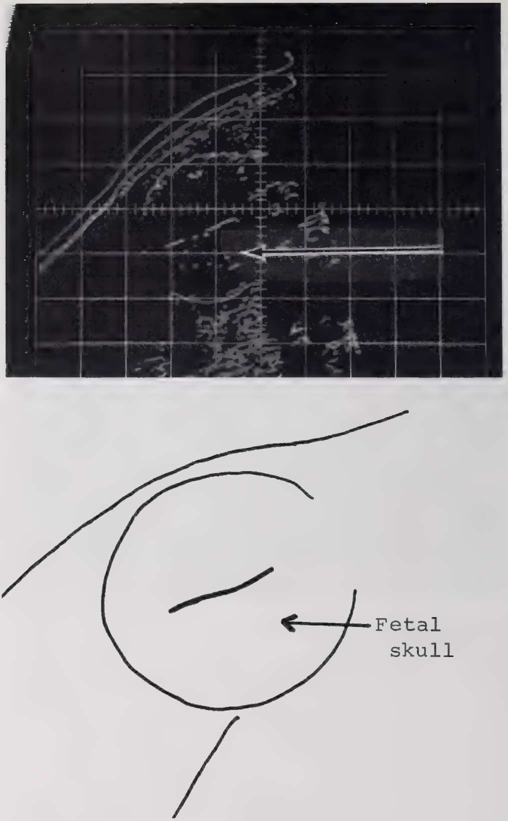

FIG. 2. Sagittal scan, term pregnancy. Note prominent midline echo.

weeks to birth (14). The incidence of false negatives (fetal heart inaudible) with the Doppler was 1–5%, usually in gravidas less than 14 weeks. In cases of uncertain IUFD, Doppler examination should not be used as the sole determinant but more as an adjunct to complete fetal sonographic scanning.

Signs of IUFD in middle and late pregnancy are based: 1) on the presence of fetal heart tones as described above for early pregnancy and 2) on sonographic visualization of the fetal head, thorax, and other body parts.

The fetal head may be seen as early as 13–14 weeks gestation in 95% of cases. Confirmatory examinations should follow at least by 1 week intervals and the head should be visualized in more than one plane (3). The sonogram of the fetal head usually shows regular round-to-elliptical contours with a prominent midline echo through which a perpendicular line defines the widest biparietal diameter (BPD) of the skull bones. The midline echo is present in 9 of 10 normal fetal head sonograms. The presence of a midline echo serves to differentiate the head from the round outline of the thorax and provides a consistant landmark for biparietal measurement. Figures 2 and 3 show the fetal skull with the midline echo. By 12 hours after IUFD, the midline echo can rarely be seen. The disappearance is believed to represent neural tissue degeneration with dissolution of the falx cerebri and interhemispheric fissure (3).

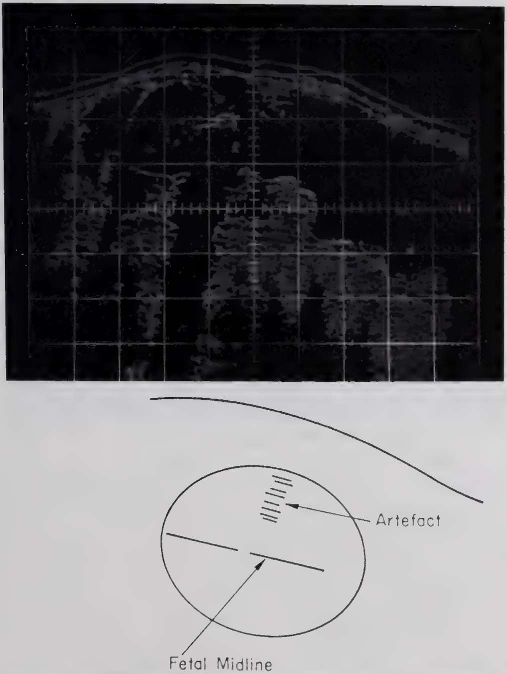

FIG. 3. Transverse scan. Fetal skull with a strong midline echo. The multiple echoes inside the near table of the skull are artifactual in nature and are probably caused by reverberations from the calvarium.

The change in the acoustical impedance of fetal tissues is a result of the penetration of amniotic fluid into epidermis, connective tissue, and deeper layers after fetal death. The edematous tissue gives a unique coarse, brush-like pattern in place of the regular well-defined fetal skull (thorax, limb parts, and other fetal tissues) outline. Coarsening of the fetal outline can be seen as early as 12 hours after fetal death (15). A double-line contour may be mistaken for coarsening of image and alone is not diagnostic of IUFD. The coarsened line of the fetal skull may be the result of tissue edema or fatty deposition in the fetus of a mother with diabetes or severe Rh-isoimmunization. The double contour of the fetal head may be produced by head movement during the scanning. However, the double contour has never persisted in a mature living fetus at rest and is probably indicative of severe tissue damage (15). Figures 4 and 5 show the coarsened and double images seen after IUFD.

Between 12 and 48 hours after fetal death, sonographic signs include the coarsened, fluffy outline of all body parts as noted above, areas normally

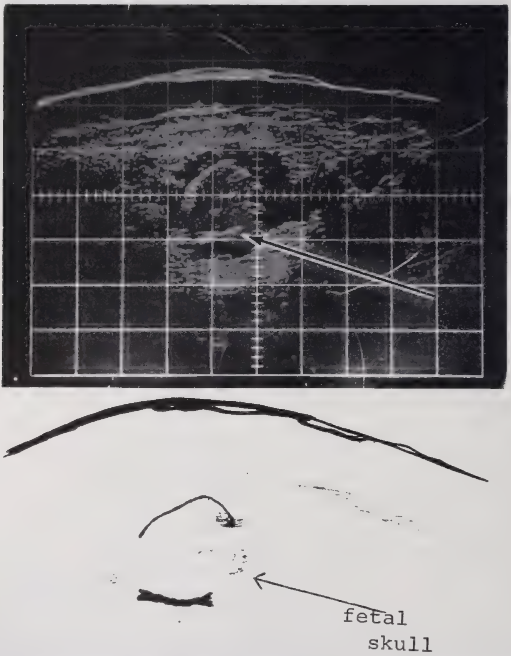

FIG. 4. Threatened abortion (22 wks). No fetal heart tone. Note increased fluffiness.

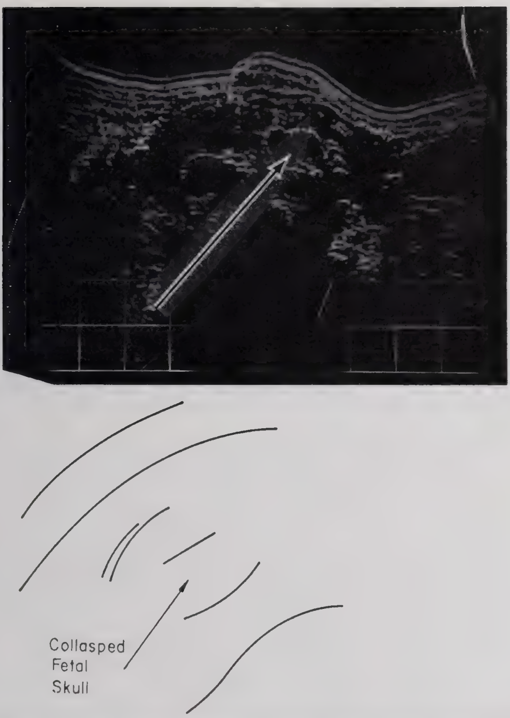

FIG. 5. Transverse scan, 30-week pregnancy. Fetal skull shows collapse, strongly indicative of fetal death.

sonolucent now dense in cross-section (especially the limb structures), and loss of the regular round fetal skull and thoracic outlines. Neural degeneration, softening of the brain with liquefaction, and collapse of the calvarium (resulting in irregular, overlapping, and flattened outlines of the fetal head) appear up to 72 hours after death. Concurrent with neural degeneration is flaccidity of skeletal musculature and collapse of fetal thorax and vertebral column. By 72 hours the fetal thoracic outline is markedly irregular, collapsing with overriding borders, and the vertebral column can no longer be recognized. By 20 days after IUFD no echoes are received either from the skull or thorax. Figure 5 shows the collapsed fetal skull. Figure 6 shows amorphous echoes within the uterus after IUFD.

Intrauterine growth failure may be demonstrated by measuring uterine size

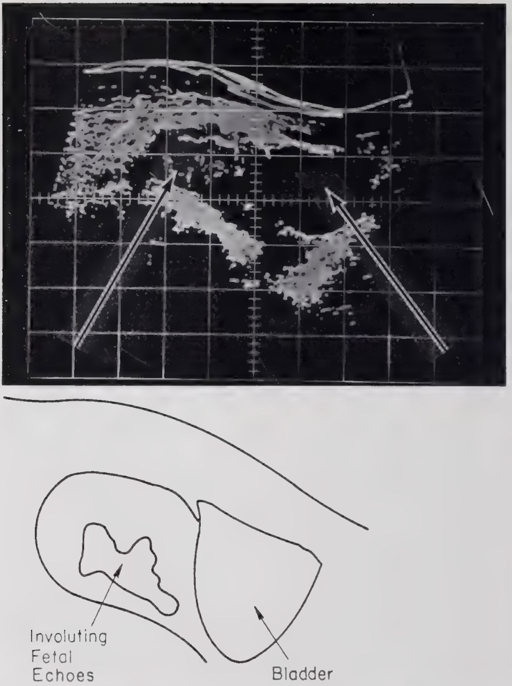

FIG. 6. Sagittal scan, 20-week pregnancy. Intrauterine fetal death. The uterus is too small for dates and contains amorphous strong echoes without a recognizable skull. Fetal heart tones were absent on Doppler examination.

on serial sonograms and may be associated with IUFD or other fetal anomalies. The two most reliable sonographic indices of fetal growth appear to be the measured biparietal diameter (BPD) and thoracic circumference, the former usually accurate to within 3 mm. It should be noted that in conditions in which BPD is critical, such as maternal diabetes, Rh-immunization, or multiple gestation, the measurement is less reliable because of potential tissue edema (3). Failure to increase BPD by at least 1 mm per week suggests retarded intrauterine growth (16). According to Thompson (16), estimates of fetal weight based on the BPD were within 600 gm in 80% of fetuses studied. Ninety-one per cent of infants studied with BPD greater than 8.5 cm and 97% of infants with BPD greater than 9.0 cm had birth weights of over 2500 grams.

Estimation of fetal growth by measurement of thoracic circumference derived from a formula by Thompson (16) was somewhat less reliable than BPD. Thompson was able to estimate fetal weight to within 300 gm in 51% and within 400 gm in 71% of cases studied. Thoracic sonographic circumference correlated with postpartum measurements to within 3 cm in 90% of cases. Using both BPD and thoracic circumference, fetal weight could be estimated to within 300 gm in 66% and within 400 gm in 82% of cases (16). In contradistinction to most American investigators, Donald believes that the BPD correlates better with fetal maturity than with fetal weight (17). In either case, BPD versus weight or maturity, a reliable estimate of fetal growth may be obtained by following BPD and chest circumference sonographically throughout pregnancy.

Intrauterine diagnosis of fetal anomalies by ultrasound includes detection of anencephaly, hydrocephaly, abnormal or absent brain ventricles, fetal ascites, and fetal polycystic kidneys (3, 5).

The presence of polyhydramnios, absent fetal head echoes, and a small circular sonodense pattern at the cephalic pole are diagnostic of an anencephalic fetus. The finding of a fetal heart tone differentiates these patterns from that of an early IUFD with obscured fetal head contours and preserved body outline.

Hydrocephalus in utero usually is diagnosed by finding a BPD greater than 10.6 cm with a relatively normal thoracic circumference (12).

Conclusion

Since 1956 diagnostic ultrasound in obstetrics has grown dramatically and provided the clinician with the ability further to appreciate location, density, and movement of previously sequestered structures. Accurate early diagnosis of IUFD or anomalies of development will influence the decision to attempt to conserve or forestall a threatened abortion or to terminate the pregnancy by early induction of labor or cesarean section. Diagnostic ultrasound may distinguish an intrauterine gestation from an ectopic pregnancy, molar pregnancy, or intrauterine myoma (2). Ultrasound has an advantage over roentgenographic methods in that 1) no calcification need be present, 2) the time interval after IUFD for x-ray signs to appear varies from 24 hours to 4 weeks, 3) there is a 25% chance that any individual case of IUFD will not demonstrate a particular x-ray sign, and 4) there are a large number of x-ray signs of IUFD that are found in the viable fetus (14).

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ENDOSCOPIC NOTES

JEROME D. WAYE, M.D., EDITOR

Surgical Problems with the Postoperative Stomach: A Technique for Meaningful Endoscopic Evaluation

Failure to empty the stomach occasionally occurs following surgical procedures performed for peptic ulcer disease. The procedure most often associated with this postoperative complication is vagotomy and gastroenterostomy (with or without resection). A method is described to evaluate this complication, circumventing the time-honored concept of watchful waiting and long-term gastric suction.

Case Report

A patient was recently evaluated who had a vagotomy and subtotal gastric resection and Billroth II anastomosis performed thirty days prior to the endoscopic evaluation. Complete outlet obstruction persisted in spite of continuous nasogastric suction. A barium meal x-ray examination revealed no filling either of afferent or efferent limbs of the anastomosis. During routine flexible endoscopy the stoma was identified and determined to be patent. Since fluids still did not pass through the stoma, the surgeon was concerned that an obstruction was present distal to the site that could be visualized with the endoscope. An endoscopic-radiographic procedure was then attempted, and liquid contrast media was instilled via a catheter placed through the endoscope directly into the afferent and efferent limbs of the anastomosis. Fluoroscopic monitoring demonstrated free flow of the contrast agent into the distal small intestine, revealing the absence of gastric outlet obstruction. It was assumed that the neurogenic vagotomy effect, coupled with postoperative stomal "spasm" or "edema" were responsible for the functional obstruction. After a subsequent week of nasogastric suction without further improvement, the patient was reexplored and in the absence of obstruction a higher gastrectomy was performed. The patient recovered; vagotomy-induced gastric atony was responsible for the problem in emptying.

Discussion

Endoscopy of the postoperative stomach may safely be performed soon after surgery if it is indicated. Hemorrhage as a complication of surgery may prompt such an examination within 48 hours of the surgical procedure. The examiner must be judicious in the amount of air instilled, but the procedure can be performed with safety. In the obstructed stomach, direct visualization of the stoma may not provide the surgeon with the answers he desires, such as whether an obstruction exists further down in the small intestine beyond the segment directly visualized by the examiner.

The differential diagnosis of postoperative gastric outlet obstruction in-

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cludes a kink of the efferent loop due to postoperative intestinal rotation, adhesions, misplaced suture, stomal edema, and a complete lack of gastric tone with pseudo-obstruction secondary to vagotomy. A definitive diagnosis is often elusive, and patients may be subjected to weeks of gastric suction hoping for the obstruction to remit. The method described herein provides a rapid and safe technique for providing a more meaningful evaluation of the problem than can be obtained either by endoscopy or radiography alone. The endoscopic portion of the procedure provides visual inspection of the stoma and ascertains if it is patent; under direct vision a thin polyvinyl catheter can be positioned through the anastomosis for the injection of contrast media. The radiographic portion of the technique permits precise localization of a site of obstruction if one is present, or allows the examiner to know immediately that free flow is obtained into the small bowel.

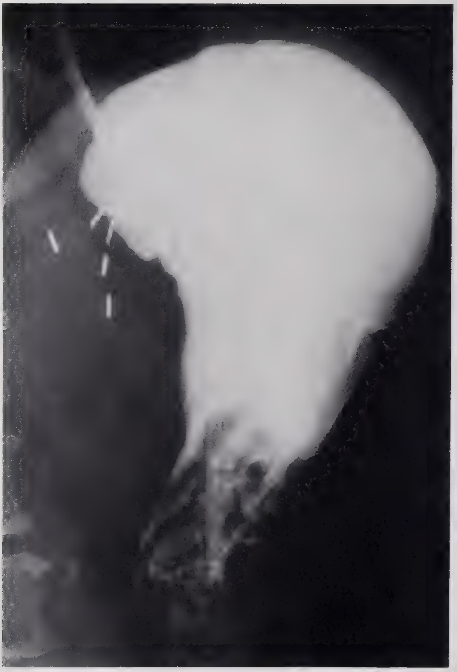

Fig. 1. Subtotal gastrectomy with nasogastric tube in place. Although the stomach was filled with barium, little flowed out through the gastrointestinal anastomosis.

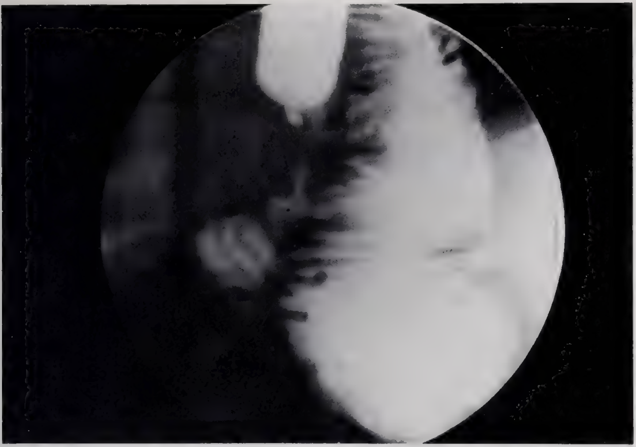

Fig. 2. The gastroscope (smoothly rounded object at top of field) is located at the anastomosis. The stoma was visualized and found to be patent, and contrast media was instilled into the afferent and efferent limbs through the endoscope. It is evident that no obstruction exists.

Summary

An endoscopic-radiographic technique has been described which is helpful in differentiating the various causes of postoperative gastric outlet obstruction.

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ENVIRONMENTAL NOTES

IRVING J. SELIKOFF, M.D., EDITOR

Zinc Protoporphyrin Determination: A Rapid Screening Test for the Detection of Lead Poisoning

By

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WILLIAM E. BLUMBERG, PH.D.†

Lead has been used since ancient times (1, 2). Its toxic effects and associated clinical manifestations have been recognized for the past two millennia. Industrial growth during the past two centuries has led to an increasing demand for lead and it is estimated that more than a million tons of lead are currently processed yearly (3). Concurrently, there has been a marked increase of the number of individuals at risk of industrial lead exposure. However, current concern for undue lead exposure goes far beyond the factory gate. Community exposure resulting from a variety of products containing lead or lead compounds, such as house paints and automobile exhausts, for example, has rendered childhood lead poisoning a major medical problem in modern society.

With regard to occupational lead poisoning, there has been a change in patterns of its reported clinical manifestations during the past few decades. This has been the result both of an increased interest in the disease as well as improvement of industrial hygiene practices. The diversity of occupations in which lead is utilized makes it difficult to evaluate the extent of lead exposure with accuracy. The lack of an effective reporting system whereby the prevalence of occupational lead intoxication might be judged further complicates the problem. "A reliable definition of the extent of risk of occupational lead exposure is unavailable" (3).

Severe, classic symptoms of acute lead poisoning are presently infrequently encountered in medical practice. Rather, effects caused by long-term, low-level exposure and so-called "subclinical" disease currently attract scientific interest. Present concepts of preventive medical practice are inconsistent with the use of manifestations of disease as a means of evaluating effectiveness of environmental control measures. There is much evidence that the cumulative effects of chronic and recurrent exposure to levels of lead insufficient to cause recognizable clinical poisoning, may nevertheless have adverse health effects (4). With this in mind it is important to keep the exposure of occupational groups and the general public to a minimum.

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The search for effective biologic test methods that are able to reveal metabolic changes in the individual before disease is established has been intensified in recent years. The usefulness of blood lead and urine lead determinations, traditionally a mainstay of biologic surveillance, has recently been questioned, primarily because they reflect recent lead absorption rather than biologic response. Furthermore, the need for a reevaluation of blood lead as an effective screening parameter among high risk populations is suggested by investigations which have shown that subclinical changes in the biosynthetic pathway of heme, as well as neurologic damage, may occur at blood lead levels hitherto regarded as "safe" (5).

Briefly, although the interference with heme synthesis by lead is a complex problem, hematologic disturbances associated with lead poisoning are perhaps the most important part of the clinical picture of this disease, especially insofar as early diagnosis is concerned. Derangements in heme metabolism constitute the basis upon which various diagnostic tests have been developed. Thus, inhibition of delta-amino levulinic acid dehydratase (ALA-D) has been shown to be a sensitive indicator of acute and chronic lead poisoning (6). Similarly, large amounts of protoporphyrin-IX can be extracted from erythrocytes formed in lead-burdened individuals. The accumulation of erythrocytic protoporphyrin is presumedly due to the inhibition of ferrochelatase, another enzyme in the biosynthetic pathway of heme. Elevated porphyrin levels in individuals with chronically elevated blood lead concentrations have been known since the 1930's (7). This so-called "free erythrocyte protoporphyrin" (FEP) can be extracted from red cells as protoporphyrin by the use of acidic organic solvents. The elaborate procedures which initially were required for FEP determinations limited the value and usefulness of this test for screening purposes. More recently, however, micromethods have been developed, with acid extraction of the blood in combination with fluorometric determination of the protoporphyrin in the extract, and these have considerably increased the practical applicability of FEP determinations (8). These procedures, however, still require laboratory facilities.

In 1974, Lamola and Yamane reported that the erythrocytic protoporphyrin is not "free" but is chelated with zinc and is converted to protoporphyrin during acid extraction (9). They also demonstrated that the zinc protoporphyrin (ZPP) can be detected by fluorometry on diluted blood with no obligatory prior extraction. Using a fluorometer with front surface illumination, they were also able to measure the concentration of ZPP in undiluted whole blood. The fluorometric principle that led to the design of a portable hematofluorometer has been described in greater detail elsewhere (10) and will only be briefly summarized here (Figs. 1–2). A drop of blood is obtained by finger puncture and deposited in the center of a glass cover slip. The cover slip

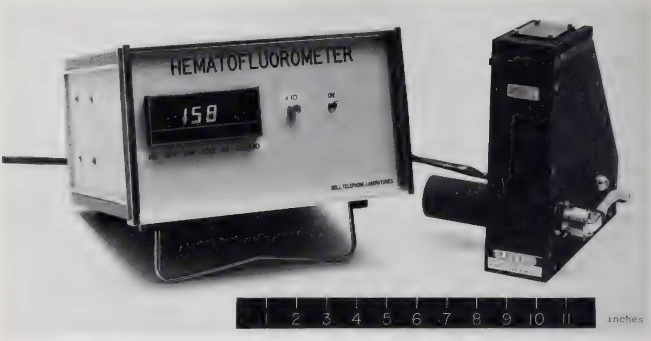
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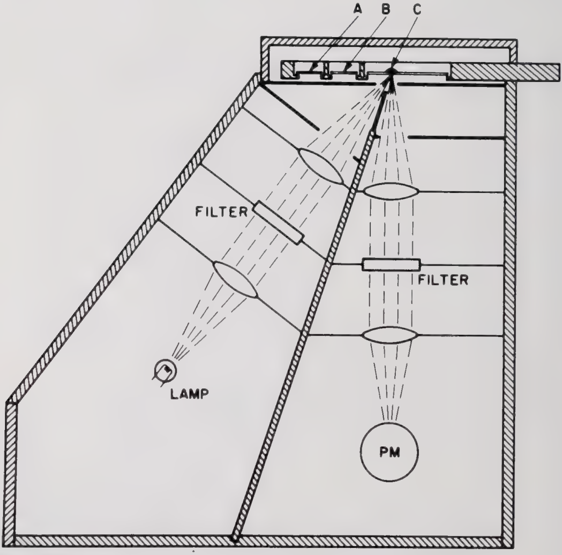
Fig. 1. The hematoflurometer—A portable field instrument able to provide an instantaneous reading of the zinc protoporphyrin concentration in micrograms per 100 ml blood.


A
B
C
FILTER
FILTER
LAMP
PM

A: blank sample
B: fluorescence standard sample
C: blood drop of examined individual on a supporting slide

Fig. 2. Schematic diagram of the optical system of the portable hematofluorometer.

is then placed on a carriage which is inserted into the apparatus. As it is inserted, the exciting light (425 nm) is focused first on a blank, then on a calibrating sample, and finally on the underside of the drop of blood, where it is completely absorbed within a few microns. As a result, the fluorescent intensity is independent of the thickness of the blood sample. Emitted light (approximately 600 nm) is filtered and detected by a photomultiplier. The instrument also contains a small dedicated computer which permits correction for background fluorescence and the calibration of the fluorescence intensity in useful units (micrograms per 100 ml) by comparison with a dye sample.

The use of the hematofluorometer has proved to be an extremely simple and rapid way to determine zinc protoporphyrin and thus to obtain a diagnostically valid index of chronic lead intoxication. The instrument has been field tested by us and we present here preliminary data showing its value in occupational surveillance programs.

Figure 3 illustrates the usefulness of ZPP determination as a means to

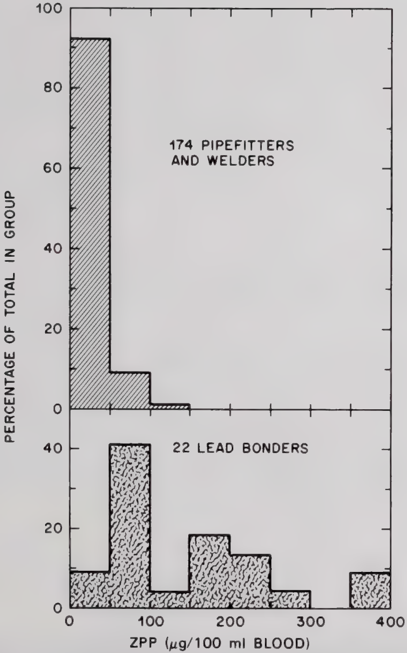


FIG. 3. Histogram showing the distribution of zinc protoporphyrin concentrations in workers with and without direct lead exposure.

identify individuals showing effects of lead exposure. In a survey of workers engaged in the construction and maintenance of nuclear reactor shielding, pipefitters and welders were seen who did not perform any operation which in itself would be associated with any appreciable lead exposure, in contrast to lead bonders. These were cutting and burning lead plates utilizing oxyacetylene torches and thereby generating considerable amounts of lead fumes. Both groups of workers were working at the same plant and indirect lead exposure to those not directly involved in handling of lead was therefore a possibility.

It was determined that most of the pipefitters and welders with ZPP concentrations ≥50μg/100 ml had experienced lead exposure during a short period preceding the examination, while working in an area where fumes containing lead were present as a result of lead-bonding work. These individuals had thus experienced indirect lead exposure, and this was reflected in a slight elevation in the ZPP concentration. However, none of those indirectly exposed had a blood lead concentration that would be indicative of increased lead absorption, unlike the results among the lead bonders, directly (and more heavily) exposed.

The correlation between ZPP (determined by the hematofluorometer) and FEP (determined by the method described by Sassa et al (8)) is shown in Figure 4. An excellent correlation was found (r = 0.98). We have also studied the correlation between ZPP and other parameters indicative of lead exposure. These data are being reported elsewhere(11).

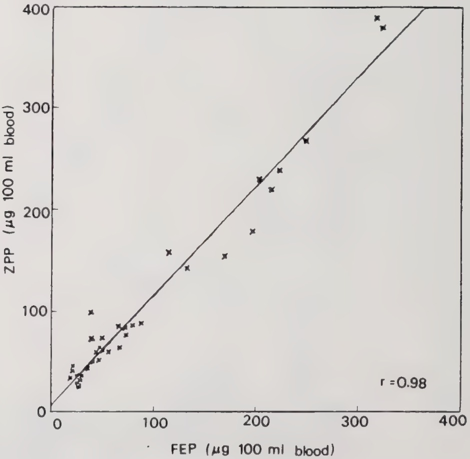


FIG. 4. Correlation between zinc protoporphyrin, determined by the hematofluorometer, and free erythrocyte protoporphyrin, determined by acidic extraction and spectrofluorometry (8).

Summary

We have found protoporphyrin determination a very useful test for the medical surveillance of lead-exposed individuals. Besides being extremely simple and inexpensive, ZPP determination with the hematofluorometer provides a useful index of lead exposure. At present, it is probably the most practical test that reflects the actual biologic effect of lead.

The introduction of the hematofluorometer into occupational medical screening programs on a large scale may contribute to the effective control of one of the oldest occupational diseases.

Acknowledgment

We are grateful to Dr. Shigeru Sassa of the Rockefeller University for performing the FEP assay.

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MEDICOLEGAL NOTES

MICHAEL G. MACDONALD, J.D. AND KATHRYN C. MEYER, J.D., COEDITORS

Defining Death

Introduction

In recent years attempts have been made in several states to establish a statutory definition of death. The debate has been heated and has been joined by a wide range of interest groups; lawyers, doctors, theologians and philosophers have all been anxious to seize the podium. Unfortunately, the discussion over the adoption of a definition of death has not been the occasion for a meaningful exchange between the medical and legal professions, but rather has precipitated yet another confrontation. Lawyers, by attempting to impose an answer by immutable legislative fiat, have ignored the strength of American jurisprudence: gradual evolution of principle through judicial decision making to accommodate the changing needs of society. Physicians, by characterizing legislative attempts at a definition of death as but another encroachment of the legal profession on the exclusive domain of the physician, have ignored the fundamental social implications of the phenomenon of death.

Background

The origin of the interest in a legislative definition of death lies in certain developments in medical technology which have taken place in the past ten years. Until recently, the exact point in time at which a person became dead had only occasional legal significance, usually in situations involving problems of the order of succession under a will. When the question arose, courts made the determination based on the then widely accepted criteria of absence of heartbeat or respiration. These criteria were not fixed in a statutory definition of death; rather they were part of what is called the common, or judge-made, law.

When advances in medical technology made it possible to keep a person's heart and lungs functioning by artificial means, questions began to be raised as to the appropriateness of expending medical resources on persons in this condition. At the same time, developments in the field of transplantation created a demand for organs which had been removed after the donor's death but before the death of the tissue, and this led to an interest in pinpointing the time of death at an earlier stage. In 1968, in response to these developments the Harvard Medical School adopted a definition of death as permanent cessation of brain function and this definition, or variations of it, was soon accepted at other medical centers. (The Mount Sinai Hospital adopted the Harvard criteria for purposes of determining the time of death in transplant cases on December 19, 1972.) However, despite this growing medical recognition

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of the brain death criteria, physicians and others remained concerned that, absent a specific statute, the older common law definition would still govern, with the consequence that a physician who removed organs for transplantation from a patient who had suffered irreversible loss of brain function (but not of heart or lung function) would find himself faced with civil or criminal liability. Interest on a nationwide level for a statutory definition of death soon emerged.

The Debate

The medical and legal professional associations have found themselves squarely in opposition on the question of the appropriateness of this type of legislation. On February 24, 1975 the House of Delegates of the American Bar Association adopted the following resolution: "For all legal purposes, a human body with irreversible cessation of total brain function, according to usual and customary standards of medical practice shall be considered dead." The accompanying committee report, while not actually calling for legislative adoption of the definition, indicated that the Committee understood that its definition would be used as a basis for statutes. Local bar associations followed suit. In July of 1975 the Public Health Committee of the New York State Bar Association issued a position paper urging legislative adoption of a definition of death. In the opinion of the Committee, the absence of a statutory definition of death created a situation of uncertainty in which doctors, fearful of possible exposure to civil or criminal liability, were hesitant to pronounce death. In addition, the Committee found that many medical examiners refuse to permit organ removal from accident or homicide victims in order to avoid the possibility that the transplant procedure will be considered the cause of death. In the opinion of the Committee, the courts had failed to keep pace with medical change in the area and legislative action was necessary to clear up uncertainties.

The American Medical Association took strong issue with the ABA's resolution and issued a statement urging the ABA to reconsider its support for the enactment of a statutory definition of death. The AMA's position focused not on the appropriateness or inappropriateness of brain death as a definition, but on the question of whether any definition ought to be embodied in a statute. In the opinion of the AMA, there were no significant legal problems relating to the time of death which would justify legislative action. The question of the time of death was a medical one calling for the professional judgment of the physician and should not be subject to mandatory control by the law. The adoption of such a statute would be "an improper intrusion by the law into the professional practice of medical physicians."

Discussion

Each of these positions, that of the bar and that of the medical profession, reflects a basic misapprehension as to the needs of the other profession. Doctors see only half the picture when they describe death as a medical diagnosis that falls within the sole purview of their profession. Whether or not a patient meets the criteria of brain death is a medical judgment, but whether brain death or some other standard is the appropriate one involves much more than that. The determination as to what point in time a person ought to be considered dead involves judgments as to the allocation of resources and relates fundamentally to a society's concept of what it means to be alive. While medicine has much to contribute to these judgments, they involve social and ethical considerations which concern other groups in society as well.

Lawyers, for their part, have made their common mistake of assuming that because a problem or confusion exists the answer is to pass a new statute. What they fail to appreciate is that medicine is an evolving science and that what constitutes a definition of death by today's standards may be inappropriate tomorrow. There is in fact currently no unanimity on the appropriateness of criteria for brain death, as recent studies have shown with respect to the conclusiveness of a flat EEG for these purposes. The difficulty with legislation is that it is inflexible and that the standards it sets become fixed and are difficult to change.

Lawyers have ignored the availability of the courts to fashion a definition of death which can be adopted and changed as society's needs changes. It is the courts who have supplied the legal definition of death when it has been needed in the past and there is no evidence that they are incapable of adjusting to recent medical developments. In fact, last summer a New York lower court ruled that the brain death standard was the appropriate one to be applied in New York. The brain death definition has been used at various medical centers for several years without legal penalty for any physicians. The strength and vitality of the common, or judge-made law has been its ability to reflect changing social values and technology and, by incremental change from case to case, to fashion solutions to problems appropriate to the situation at hand. There is no reason to believe that the common law cannot currently deal with the question of death in this fashion and in so doing be responsive to the needs of physicians as well as reflective of those of society as a whole.

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OPHTHALMOLOGIC NOTES

KEITH M. ZINN, M.D., Editor

Rubella-Associated Retinitis in an Adult: Report of a Case*

By

CLAUDE GERSTLE, M.D. and KEITH M. ZINN, M.D.

Rubella is one of the most frequent causes of disseminated chorioretinitis in the fetus. Although cases of congenitally acquired rubella may be progressive in the neonate (1), there have been no reports of postnatally contracted chorioretinitis caused by rubella. We have recently seen a patient with disseminated chorioretinitis of acute onset which was temporally related to a concurrent rubella infection as demonstrated by immunologic criterion.

Case Report

A 34-year-old Negro woman was evaluated for a complaint of decreased vision of two days duration. She had been in good health until two weeks earlier when she had an upper respiratory infection without associated headache or conjunctivitis. She had been given Norgesic® for symptomatic relief.

Course: Three days later, the patient returned with best corrected vision of 20/300 in each eye. The patient had no pain or photophobia. The anterior segments were clear and unremarkable. The intraocular pressures were normal. The pupils were equal, round, and reactive to light and accommodation. Funduscopic examination revealed large areas of bullous retinal detachment involving the entire posterior pole (Figs. 1A and 1B). Several areas of underlying pigment epithelial detachment were also evident. In the preretinal vitreous, white cells were present as well as a single white gliotic mass approximately of 1/4 disc diameter in size. The gliotic lesion was located in the infero-temporal quadrant just below the left macula. History and physical examination were unremarkable, except for a negative history of rubella or recent exposure to this agent.

Laboratory studies: Complete blood count, sedimentation rate, 18-component screen, and chest x-ray studies were normal. Serum protein electrophoresis revealed moderately elevated beta globulins. Spinal fluid examination and audiograms were normal. An intermediate strength PPD skin test was negative. Antibody titres for herpes, CMV, toxoplasmosis, adenovirus, and histoplasmosis were negative. The rubella titre was abnormal at 1:160, and the measles titre was 1:128. Two weeks later, the measles titre remained at 1:128 whereas the rubella titre rose to 1:640. Two months after the acute phase, the rubella titre was 1:32. Conjunctival viral cultures were not done.

Hospital course: The patient was admitted to the hospital for observation. Fluorescein angiograms revealed multiple areas of choroidal fluorescein leakage. There was no leakage from the retinal

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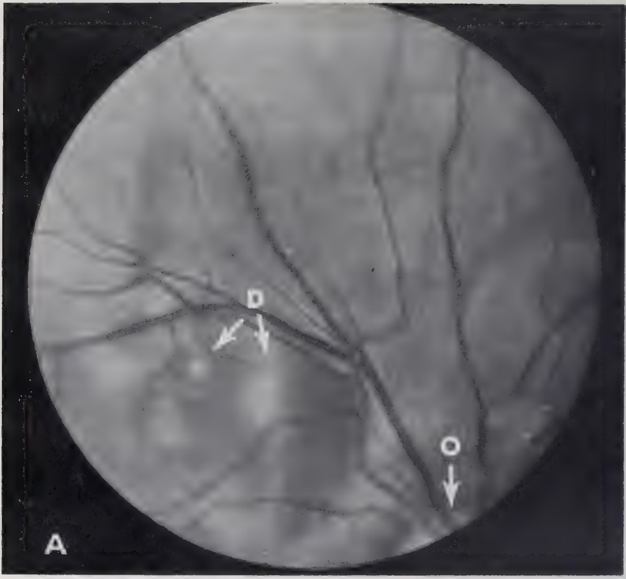

FIG. 1A. Fundus photo (right eye) of the region supero-temporal to the optic nerve head (O). There are multifocal areas with serous detachments of the neurosensory retina (D) and the pigment epithelium.

vessels. The underlying choroidal blush appeared abnormal with partially obscured fluorescence. In the later portions of the angiogram, these spots enlarged with obvious leakage into the subretinal spaces (Figs. 2–5). Four days after admission, the patient was subjectively unchanged, but was noted to have fine cells in the anterior chamber. Treatment with cycloplegics and topical Decadron® was begun. Vision rapidly improved to 20/20 in both eyes, within three weeks after admission and, although some areas of bullous detachment remained, the steroids were discontinued in view of the clear anterior chamber. Three months later, the vision was 20/20 in both eyes, with the patient's original correction. The retina was flat, but several areas of pigment epithelium depigmentation were present. Repeat fluorescein angiograms were normal except for the areas of depigmented pigment epithelium.

Discussion

The proven etiologies of chorioretinitis causing nonrhegmatogenous retinal detachments include cysticercus, nocardia, toxocara, toxoplasma, herpes zoster, and sarcoid (2–4). There are also a number of fairly well-defined syndromes of seemingly inflammatory origin which can manifest nonrhegmatogenous detachments. Vogt-Koyanogi-Harada disease (VKH), Behçet's

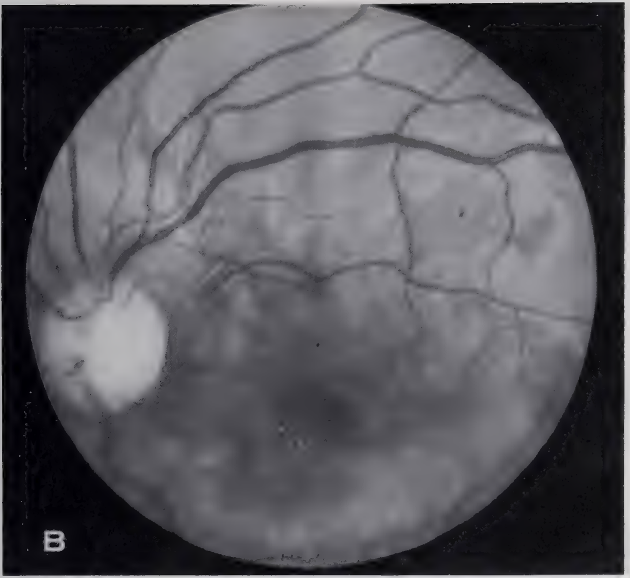

FIG. 1B. Fundus photo (left eye) of the disc and macular region. There are multifocal areas with serous detachments of the neurosensory retina and the pigment epithelium.

disease, the retinal pigment epitheliitis of Klein and Deutman, and, possibly, uveal effusion and central serous retinopathy, fall into this group.

The VKH syndrome consists of chorioretinitis with exudative retinal detachment, usually occurring in darkly pigmented persons 20 to 40 years of age. There is often a cerebrospinal fluid pleocytosis with meningeal irritation at some point in the disease; and dysacusia, vitiligo, and poliosis often ensue later in the course of the disease. There are many clinical variations, both in the severity and type of symptoms present. The case under consideration has many similarities to VKH disease. The patient is of the correct race and age. The duration of the detachment was shorter than most cases of VKH, but similarly brief forme frustes of the syndrome have been included in published case series (5). The fluorescein angiogram is typical of disseminated choroiditis or pigment epitheliitis. The prompt leakage from choroidal vessels at an early stage of the angiogram with progressive subretinal extension of fluid points to a diffuse pigment epithelial dysfunction; whether the choroid itself is involved is debatable. In acute placoid pigment epitheliitis, the involved cells

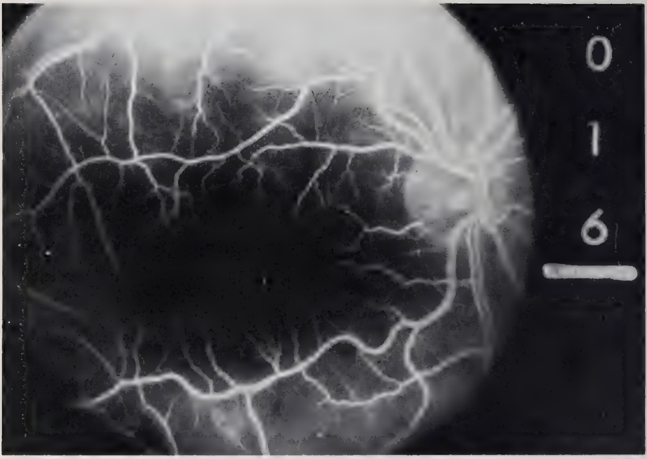

FIG. 2. Fluorescein angiogram (right eye). Focal areas of choroidal fluorescein leakage in the region superior to the macula in the early arterio-venous phase.

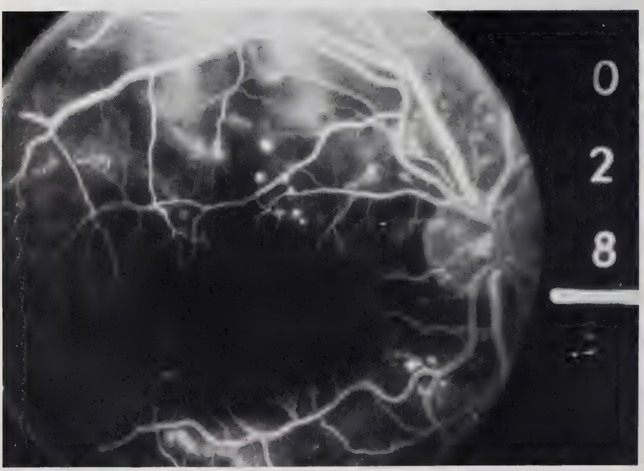

FIG. 3. Fluorescein angiogram (right eye). New multifocal sites of leakage at the level of the retinal pigment epithelium along with pooling of fluorescein in the subretinal space just superior to the macula.

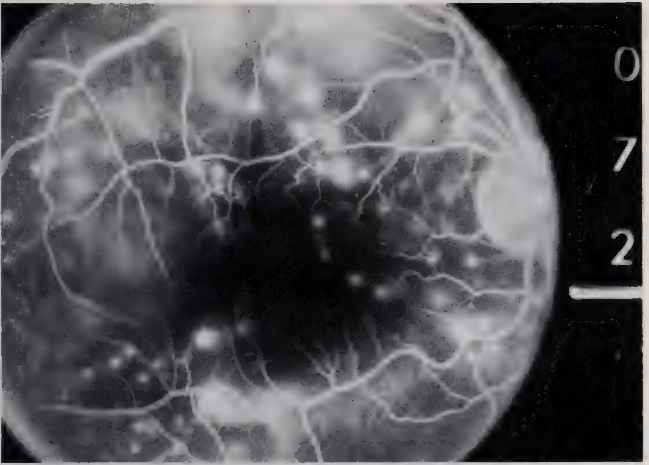

FIG. 4. Fluorescein angiogram (right eye). Continued leakage of fluorescein from foci of damaged retinal pigment epithelium resulting in increased pooling of dye in the subretinal space. This dye accumulation partially obscures the choroidal vascular pattern.

swell, obscuring the underlying choroidal fluorescence (6), but serous detachments do not occur. In the acute pigment epitheliitis of Krill and Deutman, angiography showed small, central, nontransmitting spots surrounded by a halo of increased transmission of choroidal fluorescence through the pigment epithelium. In one case, there was leakage through the affected area into the subretinal space. This is a self-limited disease entity in otherwise healthy young (20- to 40-year-old) patients manifesting symptoms of decreased vision and metamorphopsia. Fundus examination shows one to four clusters of small black spots deep in the retina. Within 6 to 12 weeks, vision returns to normal with some residual disorganization of the pigment epithelium remaining. On the other hand, with disseminated choroiditis, infiltration of the choroid also causes diminished choroidal fluorescence, as was seen in the areas of the angiogram between the spots of leakage through the pigment epithelium. This diminished fluorescence is probably a result of thickening and infiltration of the choroid (8). It seems difficult to distinguish clinically whether the diminished choroidal blush is caused by an edematous pigment epithelium or an infiltrated choroid.

The rise in antibody titres to rubella in this patient, while not conclusive proof that this was the etiologic agent, prompts some interesting speculations. Rubella infection in utero produces a diffuse viral infection. Pathologically, in the eye, the major site of destruction is in the pigment epithelium, although the cornea and lens also show active infection (8, 9). Without the pathologic

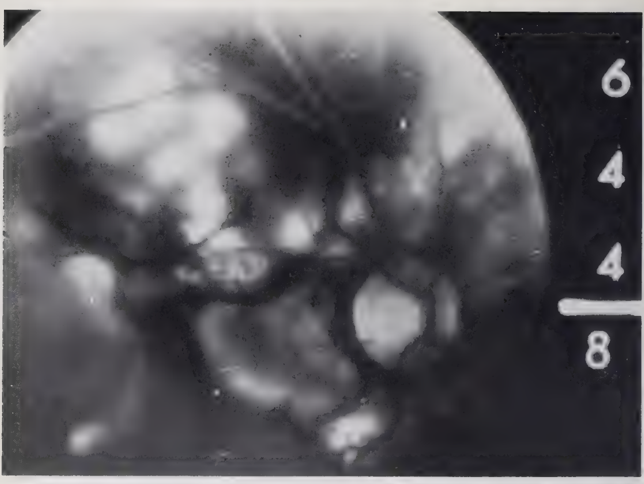

FIG. 5. Fluorescein Angiogram (right eye). Late phase demonstrating considerable dye accumulation in the subretinal and subpigment epithelial spaces in regions of diseased pigment epithelium.

tissue to demonstrate the presence of the virus within the pigment epithelium, a more conclusive determination of the pathogenesis of this disorder is not possible at the present time.

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NEUROLOGIC-NEUROSURGICAL NOTES

JOHN LUSINS, M.D. AND MARTIN H. SAVITZ, M.D., COEDITORS

Spinal Epidural Hematoma: Report of Two Cases

By

MARTIN H. SAVITZ, M.D., RAFAEL QUINONEZ, M.D., SIDNEY A. HOLLIN, M.D.,
AND S. SHELDON KATZ, M.D.

Cord compression in the presence of nondeforming fracture due to extradural hemorrhage in the spinal canal is apparently a rare entity, since references in the world literature are few in number (1, 2). Hematorrhacis externa may also occur as a result of straining (3), bleeding diathesis (4), preexisting hemangioma (5), or minor trauma (6). The purpose of this paper is to report two cases of spinal cord injury resulting from epidural hematomas following vertebral fractures that did not compromise the spinal canal.

Case Reports

Case 1: A 12-year-old boy fell down one flight of stairs and struck his dorsal spine on a railing. He was able to climb two flights of stairs to his place of residence. After sitting in a chair for twenty minutes, he experienced the sudden onset of numbness in both legs and could not stand up. His parents brought him to medical attention without delay. On admission to the emergency room, he complained of unremitting pain in the middle of his back.

Physical examination revealed a small superficial abrasion overlying the sixth thoracic spinous process. Neurologic testing was performed by a neurologist and two neurosurgeons. Total flaccid paraplegia and a T6 sensory level were demonstrated. Priapism was present.

The patient was given dexamethasone 10 mg intravenously and continued on 4 mg every six hours. X-rays of the dorsal spine showed mild compression fractures of the fourth, fifth, sixth thoracic vertebrae; there was no impingement on the spinal canal. Lumbar puncture produced clear and colorless cerebrospinal fluid; no cells were present, and the sugar and protein content were within normal limits. The Queckenstedt test revealed a manometric block. An emergency myelogram confirmed the complete block at the 78 level.

The patient was taken to the operating room and underwent a decompressive laminectomy from 78 through 74. As the bone was removed by rongeur, formed blood clot was visible in the epidural space. The intact dura was not noted to pulsate until 5 cc of epidural hematoma had been removed and the laminectomy had been completed.

Immediately following surgery, voluntary contractions of the hamstring muscles were observed. There was partial return of all sensory modalities in the lower extremities. On the first postoperative day, the plantar reflexes could not be elicited; and a level of hypesthesia was found up to the T8 dermatome. On the second postoperatively day, he was able to move his right quadriceps femoris and extensor digitorum brevis; bilateral Babinski signs were present. On the third postoperative day, he could flex and extend both knees and ankles. The steroid doses were then tapered. On the fifth postoperative day, voluntary movements in all muscle groups of both lower extremities could be tested.

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Physiotherapy was instituted. At the end of the first postoperative week, the patient was fitted with a Knight spinal brace and mobilized. Anal sphincter and bladder function were normal. The incision healed well, and he was discharged home at the end of the third postoperative week. Two years after the spinal cord injury, his neurologic examination was completely within normal limits; x-rays of the thoracic spine revealed mild scoliosis.

Case 2: A 58-year-old woman was involved in a moving vehicular accident and suffered multiple injuries including facial lacerations, blunt trauma to the anterior chest wall and abdomen, and fractures of the right forearm. On arrival at the emergency room, she was unable to remember the details of her accident and complained of severe pain in the interscapular area.

Physical examination revealed multiple superficial lacerations of the frons and buccae, a large area of ecchymosis over the sternum, generalized tenderness of the abdomen, and hematuria. Neurologic testing was performed by a neurologist and found to be completely within normal limits except for amnesia about the events of her trauma. X-rays of the skull, cervical spine, and thoracic spine were negative for fracture. X-rays of the right forearm revealed fractures of the distal radius and ulna which were immobilized in a plaster cast by an orthopedic consultant.

The patient was admitted to the hospital; her vital signs, level of consciousness, and pupillary reflexes were monitored hourly. Approximately twelve hours after admission, her blood pressure dropped from 130/90 to 100/70; but stabilized with intravenous fluid therapy. She complained of weakness and numbness in her hands and legs. Motor testing revealed total flaccid paraplegia; extension and flexion of the shoulders, extension of the elbows, and flexion of the wrists were the only voluntary movements in the upper extremities. Sensory examination demonstrated complete anesthesia below the C7 dermatome. No deep tendon reflexes could be elicited except the triceps jerks.

The patient was given dexamethasone 10 mg intravenously and continued on 4 mg every six hours. Repeat x-rays of the lower cervical spine failed to demonstrate any fracture. An emergency myeologram was performed by a neurosurgical consultant through a lateral C1-2 spinal puncture and showed a complete block at the C8 level. The cerebrospinal fluid was clear and colorless; no cells were present, and the sugar and protein content were within normal limits.

She was taken to the operating room and underwent a decompressive laminectomy from T1 through C6. As the muscle was stripped from the spinous processes and underlying laminae, fractures were visualized on both sides of the laminar arch of the seventh cervical vertebra. When the bone was removed by rongeur, formed blood clot was visible in the epidural space. The intact dura began to pulsate minimally after the laminectomy had been completed and 4 cc of epidural hematoma had been removed.

Immediately after surgery, the neurologic examination was unchanged. The steroid therapy was tapered over a five-day period. The incision healed well. One year after the spinal cord injury, there was no improvement in motor power or sensation.

Discussion

The initial symptom in reported cases of spinal epidural hematoma was almost invariably severe pain in the back at the level of hemorrhage; numbness and weakness were delayed from minutes to days. X-rays of the spine rarely revealed vertebral fracture. Manometric testing following spinal tap often demonstrated a complete block. Myelography confirmed the level of the block, but the diagnosis was not made until surgery or postmortem examination.

The vertebral venous system consists of a valveless plexiform network which is connected above with the cranial dural sinuses, below with pelvic veins, and in the neck and trunk with the azygous and caval systems. The potential for spinal cord compression due to hemorrhage from epidural veins is somewhat decreased by the relative difference in cerebrospinal fluid and venous pressures. The exact source of bleeding was established in only two reported cases (5, 7).

The indications for laminectomy following spinal cord injury are by no means well agreed upon (8). Some neurosurgeons are of the opinion that the patient who has complete blockage of the subarachnoid space on the Queckenstedt test, whether the neurologic deficit is immediate, complete, or incomplete, should be explored as soon as possible. Most authorities advise neurosurgical intervention if any progression in neurologic signs and symptoms is documented.

Summary

Two cases of spinal epidural hematoma are reported. The various etiologies of hematorrhachis externa are discussed. The results of diagnostic procedures and neurosurgical exploration are presented.

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UNUSUAL PROBLEMS IN SURGERY

A. ROBERT BECK, M.D., EDITOR

A Technique for Hepatic Resection in Infants

Much has been written concerning the details of hepatic surgery in adults. It is the intent of this paper to report the experience gained in the resection of tumors of the right lobe of the liver in three infants, and to describe some differences in technique from those used in adults.

Technique

As in adults, adequate exposure of the liver is essential. Although most authors state that a thoraco-abdominal incision is mandatory (1–4), this is not the case in infants. A modified bilateral subcostal incision, as illustrated in Figure 1, allows displacement of the entire right lobe of the liver into the operative field following division of the hepatic ligaments. Excellent exposure of the hepatic veins is facilitated by the extreme pliability of the ribs. A similar incision has been found useful in the resection of large Wilm's tumors and neuroblastomas. In all of our cases a sufficient margin of normal tissue was present between the tumor and the interlobar plane so that the gallbladder could be preserved. The line of resection was determined, and a series of interlocking through and through 2/0 chromic catgut mattress sutures were placed 1 cm to the left of this plane. Branches of the hepatic artery and the bile ducts were not individually ligated. The liver was divided 1 cm lateral to the suture line using electrocautery (Fig. 2). Very little bleeding was encountered. Excellent hemostasis was obtained in all cases simply by compressing the liver parenchyma between the thumb and index finger. The pressure was then slowly released, and the bleeders identified and carefully suture ligated. Because of the small size and soft consistency of the infant's liver, this method appears to be superior to the finger fracture technique described by Lin (5). In all cases, adequate drainage of the resected liver edge was employed. The biliary tree was not decompressed.

Case Reports

Case 1: A male infant was born following an uncomplicated pregnancy and delivery. Shortly after birth, a 4 x 5 cm right upper quadrant abdominal mass was felt (Fig. 3). The infant was otherwise normal. On the third day of life the infant developed signs and symptoms of congestive heart failure. Arteriography was performed through an umbilical artery and a large vascular tumor of the right lobe of the liver was demonstrated.

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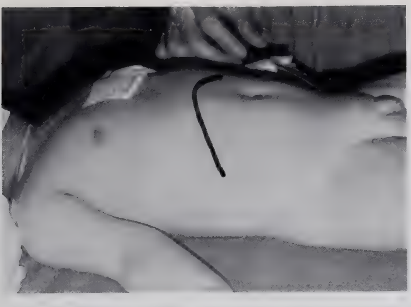

FIG. 1. Modified bilateral subcostal incision.

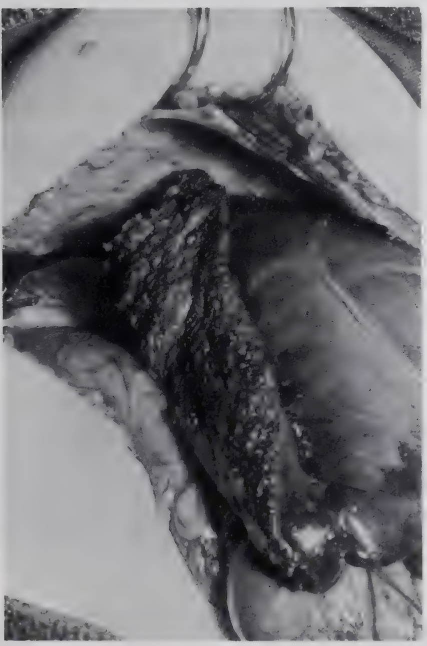

FIG. 2. Liver edge following resection of tumor. Note line of interlocking catgut sutures proximal to the line of resection.

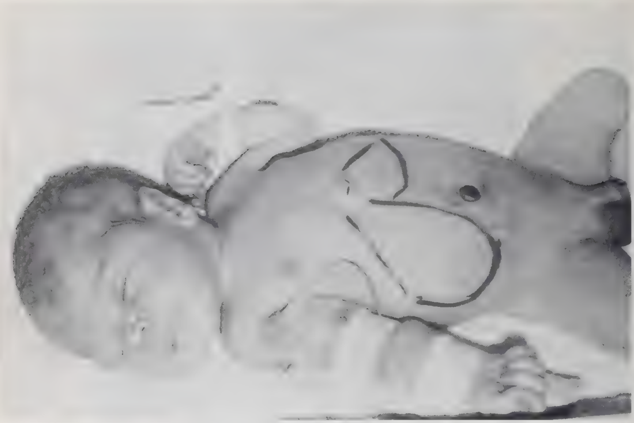

FIG. 3. Vascular hamartoma of the right lobe of the liver causing congestive heart failure.

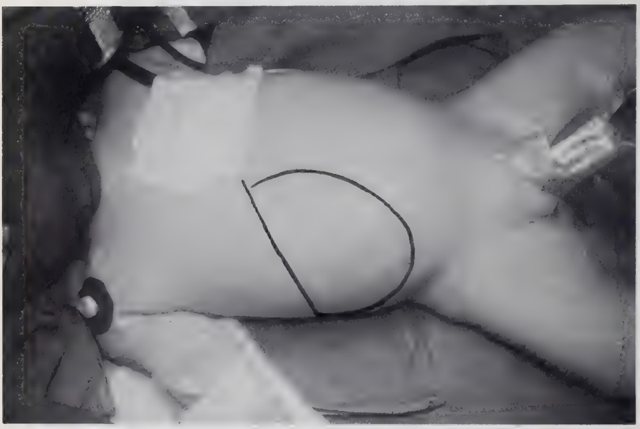

FIG. 4. Hemangioma of the right lobe of the liver.

At surgery, a large, irregular vascular hamartoma involving the entire right lobe of the liver was found. It was resected with most of the right hepatic lobe, but the gall bladder was preserved. Blood loss was estimated to be 25 cc. Postoperatively the patient did well. The hematocrit and plasma proteins remained stable and the congestive heart failure cleared rapidly.

Case 2: A full-term female was born after a normal gestation and delivery. A 7 x 10 cm abdominal mass was noted immediately after birth. The mass, which was firm and occupied the entire right side of the abdomen, extended to the iliac crest (Fig. 4). The platelet count was 30,000 per cu mm. A liver scan demonstrated a large, well-demarcated defect occupying almost the entire right lobe of the liver.

At ten days of age, a right hepatic lobectomy was performed and a solitary hemangioma of the right lobe of the liver was excised. The estimated blood loss was 75 cc. The postoperative course was marred by a partial small bowel obstruction, which was relieved by nasogastric suction. The patient was discharged three weeks following surgery.

Case 3: An eight-month-old, previously healthy male infant was admitted with a 24-hour history of fever, vomiting and abdominal distention. On physical examination, the patient had signs and symptoms of diffuse peritonitis. The white blood cell count was elevated and the hemoglobin was 9.0 G%. Roentnograms of the abdomen were nonspecific. The patient was anesthetized, and the abdomen was palpated prior to surgery. A 10 x 15 cm mass was felt in the right upper abdomen. Laparotomy was performed, and a large embryonal hepatoblastoma of the right lobe of the liver was found. The tumor had perforated on its anterior surface, and the peritoneal cavity contained 100 cc of blood. A right hepatic lobectomy was performed, leaving the gallbladder intact. One hundred seventy cc of blood was given during surgery. The patient did well, and required no further transfusions. He is now receiving chemotherapy one year following surgery.

Summary

A technique for resection of tumors of the right lobe of the liver in infants is described, and some of the differences from those used in adults are briefly discussed.

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THE STUDENT'S CORNER

SHARON WILKINSON, B.A., Editor

This column is a forum for medical students to comment on issues concerning medical school and medical education. The editor of this column is a fourth-year medical student and elected student representative at Mount Sinai School of Medicine of The City University of New York. Mount Sinai School of Medicine students are invited to take advantage of this opportunity to reach a wide and influential readership. Medical students from other schools are also invited to contribute.

Contact Ms. Wilkinson through Mount Sinai School of Medicine, The Annenberg Building, Room 13-98A, Fifth Avenue and 100 Street, New York, N. Y. 10029. If you do not have time to write an entire column, you can still contact Ms. Wilkinson and share your thoughts on a particular topic which perhaps can be incorporated into a future column.

David A. Dreiling, M.D.
Editor-in-Chief

Primary Care and Medical Education

GEORGE VITEK, S.B.*

Many of the problems in health care today deal with the delivery of primary care. Dr. Jesse L. Steinfeld, former United States Surgeon General, stated, "In recent years, American medicine and the American public have come to the realization that, despite the fantastic progress in biomedical research, large numbers of our citizens are receiving inadequate or, in some cases, virtually no health care" (1). Dr. Barry Stimmel recently wrote, "Available data indicate that the number of physicians engaged in primary care not only has not kept pace with the increase in the general population but, in terms of absolute numbers, has actually decreased from 1965 to 1972" (2). One reason for this problem lies in medical education. The medical student's exposure to medicine is traditionally limited to large university-medical center complexes. Students are usually exposed to patients in secondary and tertiary care institutions. Research-oriented physicians with expertise in a very specific area of medicine become the major role models for students. This limited exposure conditions the student's ideas concerning the issues and needs of medicine, and it is only natural that his or her goals become directed toward specialization rather than primary care.

The need exists to expose medical students to the practice of primary care. The future primary care physician will practice with a team of other physicians, nurses, and allied health workers. In addition to providing services, the physician will be concerned with the management and the organizational delivery of services (3). To prepare the medical student for the delivery of primary care certain specific educational needs can be set forth. First, the student needs knowledge about team care and skills in interpersonal relations and group processes. Second, the student requires knowledge of health care organization and financing and skills in health care management. Third, the student needs experience in community health planning and community health resource utilization.

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* George Vitek is a second-year student at Mount Sinai School of Medicine of The City University of New York. He worked in a health fieldwork program in the Department of Urban Studies and Planning at the Massachusetts Institute of Technology and is presently in a Department of Community Medicine elective with the East Harlem Tenants Council in the development of the Taino Towers Neighborhood Health Center.

Fieldwork is one important teaching method for preparing students interested in primary care. Fieldwork places a student outside the traditional classroom setting and in an existing service program where he or she deals with problems and issues of importance to the organization. The setting provides role models and personal role development, knowledge of group dynamics, and understanding both of provider and consumer attitudes, ideas, and expectations of medical care. The work content provides skills and knowledge in primary care services, health care management and planning, and community population analysis.

In terms of an educational experience, fieldwork incorporates several key ingredients for effective learning. These are relevance, individuality, and active participation. Relevance is important in motivating one to learn and to do further work on one's own. The responsibility of working on problems affecting continuing programs provides stimulation and personal satisfaction. Relevance varies for each student, and fieldwork provides the opportunity to tailor the experience to the individual. Each student brings to his or her project a different set of abilities, previous knowledge and experiences, and educational needs. Field experience can also be tailored to the educational level of the student. Before clinical skills are developed, the content of the experience can focus on health care delivery. After clinical skills have developed, the student can then concentrate on the aspects of clinical primary care. Finally, learning through fieldwork is largely based on the value of "learning through doing." The student is active in his or her own education rather than a passive recipient.

Medical education has a role in teaching and exposing students to issues and knowledge in health care delivery and primary care practice. Fieldwork in primary care offers a broadening experience in an area of important need and challenge.

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	Drugs																
Gram Positive Organisms	Ampicillin	Carbenicillin	Cephalothin (Keflin, Kefzol. Keflex)	Chloramphenicol	Erythromycin	Gentamicin	Clindamycin	Kanamycin (Neomycin)*	Penicillin	Oxacillin (Prostaphlin) Methicillin (Staphcillin)	Colymycin	Streptomycin	Tetracycline	Vancomycin	Sulfonamide	Nalidixic Acid (Neggram)**	Nitrofurantoin (Furadantin)**
Staphylococcus aureus	4	—	1	2	2	1	2	2	4	1	—	—	2	1	4	—	—
Enterococcus (S. faecalis) (S. faecium)	2	—	5	2	3	3	6	6	2	6	—	—	5	2	5	—	—
Beta Hemolytic Streptococcus	1	—	1	1	1	—	1	6	1	2	—	—	3	1	6	—	—
Streptococcus Viridans	1	—	1	1	1	—	1	6	1	2	—	—	3	2	5	—	—
Anaerob. and Microaeroph. Streptococci	1	—	1	1	1	—	1	5	1	1	—	—	4	1	6	—	—
Pneumococcus	1	—	1	1	1	—	1	6	1	1	—	—	2	1	4	—	—
Clost. perfringens	1	1	2	2	1	5	2	6	2	—	—	—	4	—	—	—	—

Gram Negative Organisms																	
Bacteroides species	5	—	5	1	3	4	2	5	4	—	—	5	4	—	—	—	—
Escherichia coli	3	3	3	2	—	1	—	2	—	—	2	4	4	—	4	2	2
Enterobacter species	5	3	5	2	—	1	—	2	—	—	2	3	3	—	5	2	2
Klebsiella species	6	6	3	3	—	2	—	2	—	—	2	3	4	—	5	2	2
Indole Positive Proteus species	5	2	5	2	—	1	—	2	—	—	6	3	5	—	5	2	2
Proteus mirabilis	2	2	2	2	—	1	—	2	—	—	6	3	5	—	4	2	2
Ps. aeruginosa	6	3	6	5	—	1	—	5	—	—	2	4	6	—	4	5	6
Pseudomonas species	6	3	6	4	—	2	—	4	—	—	3	4	5	5	4	5	6
Salmonella	3	—	2	1	—	1	—	1	—	—	—	4	3	—	5	—	—
Serratia marcescens	6	4	6	4	—	1	—	4	—	—	5	4	5	—	5	4	4
Shigella	1	1	1	1	—	1	—	1	—	—	1	—	4	—	4	—	—
Hemophilus influenzae	1	1	1	1	1	1	5	1	2	—	—	1	1	2	2	—	—

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** Urine isolates

(—) Insufficient number of strains tested

Prepared by: Edward J. Bottone, Ph.D., Director of Microbiology and Bruce A. Hanna, Ph.D., Assistant Microbiologist

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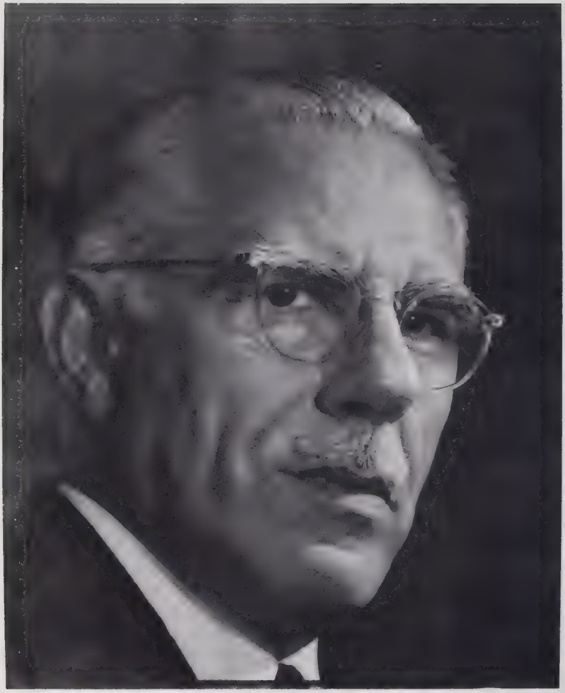


RALPH COLP, M.D.

DEDICATION

Dr. Ralph Colp belonged to the Age of the Giants. He joined the Mount Sinai staff during the era of the Big B's—Berg, Buerger, and Beer—a difficult trio to follow. Yet Colp and his contemporaries, John Garlock, Harold Neuhof, Abraham Hyman, and Leon Ginzburg, carried on the tradition of greatness, each in his own field of specialization.

Doctor Colp was a man of enormous complexity. Like Eve, he had three faces—Colp the Man, Colp the Teacher and Investigator, and Colp the Surgeon. These manifestations, differing fundamentally, logically interfaced to produce the Man underestimated by many, misunderstood by some, and intimately known by only a few.

Colp the Man had a hard exterior, somewhat aloof and gruff at first. Yet beneath this facade was a debonair character, a bon vivant who cut a handsome and charming figure in the social, intellectual, and professional life of his time. Ralph was a meticulous dresser, he was punctilious, extremely well mannered, and scrupulously honest in his dealings. He demanded these features in those he would befriend. Yet Ralph was not effete—far from it. If he did not respect you, you knew; if he disliked or distrusted you, he made no bones about it; if he opposed you, you were informed of his opposition. Above all, if he once espoused your cause, you were never betrayed. The battle might be bloody, but, like Luther, there he stood. Yet, despite his strength and determination, Ralph could be reasoned with, could be made to change his mind, and could be made to admit his error.

Doctor Colp loved the finer things of life. Gracious living was important to him. Elegance was his standard and culture was worshipped by him. In many ways Ralph Colp measured up to the Victorian concept of Father Image—stern, dignified, honest—but he could be kind and he could be generous!

The scholastic accomplishments of Ralph Colp have been unrecognized generally. He was the first of the Giants to recognize the importance of basic science to his clinical development. In early 1940 he was instrumental in setting up Franklyn Holland, a Ph.D. gastrointestinal physiologist in a GI research laboratory that still exists today. Frank and the young surgeons who rotated through his laboratory, e.g., Eugene Klein, Edward Jemerin, and Vernon Weinstein, elucidated much of the physiology of gastric secretion and vagal effects on secretin that forms the basis for the Hollander-Jemerin test as well as the treatment of duodenal ulcer. Long before the published work of Dragstedt, Ralph Colp reported 2 patients in whom vagesection was unsuccessfully employed to control the hypersecretion of duodenal ulcer!

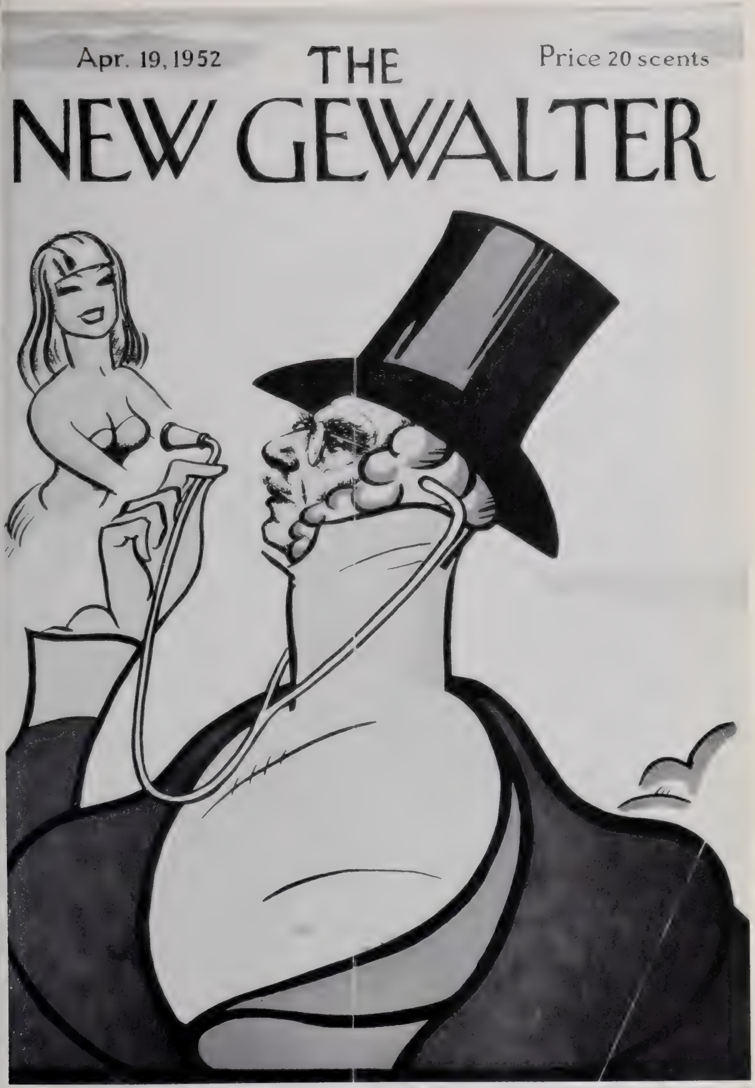
Doctor Colp's interest in pancreatic disorders started early in his career and persisted until retirement. He established a Colp Fellowship for research in this area. In succession, this Fellowship was held by Henri Doubelet, David A. Dreiling, and Augusto Paulino Netto. In addition, Ralph founded the Pancreatic Research Laboratory at Mount Sinai, funded it, and induced me to run it in 1948. Its activities continue today.

Ralph Colp the Surgeon was a great teacher. Having served for many years as a morphologist, his anatomic knowledge was so precise that he was able to perform his operations by sharp dissection with exceptional speed and dexterity. There never was a wasted motion. The assistants were fully utilized in a team effort. Ralph had a talent for delegating authority, but for this he demanded responsibility. Woe to his surgical associates who could not satisfy this sine qua non.

As a physician Colp had an inquiring mind. His interest in basic science, sphincterotomy, and vagesection exemplified an innovative, pioneering trait. He did much to encourage investigation, not only by establishing and funding laboratories and creating fellowships, but also by creating prizes for the best published papers in *The Mount Sinai Journal of Medicine* and by instituting lectureships to bring the most sophisticated medicine to Mount Sinai.

Perhaps all that Ralph Colp was is best illustrated by the frontispiece of *The New Gewalter*. This caricature epitomizes Ralph Colp.

David A. Dreiling, M.D.
Second Ralph Colp Fellow,
Experimental Surgery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COLP LECTURESHIPS

Pursuit of the Elusive: Adventure in Quest of a Specific Test for Pancreatic Amylase*

J. EDWARD BERK, M.D. AND LOUIS FRIDHANDLER, PH.D.

The prevalence and clinical importance of diseases of the pancreas make it highly desirable to be able to identify pancreatic disease early and easily. Serum and urinary amylase determinations are the simplest diagnostic approaches currently available and are widely used. Measurement of total amylase activity, however, supplies only limited information and may even be misleading because of certain inherent shortcomings (1). Chief among the latter are: 1) the relatively rapid rise and fall of serum amylase activity in acute pancreatitis; 2) the dependence of elevation in serum amylase activity on the amount of acinar tissue still functioning and unaffected by disease processes that involve the pancreas; 3) the occurrence of hyperamylasemia in various nonpancreatic disorders; and 4) the rise in serum amylase activity that may follow the administration of analgesics (2). Furthermore, assay of total amylase in the serum provides no information regarding the tissue origin(s) of the enzymatic activity being measured.

At one time unsettled (3), it is now well recognized that amylase is not a single entity but is composed rather of a variety of isoenzymes that arise from different tissues. Existing knowledge of the behavioral characteristics of the amylase isoenzymes was admirably summarized in 1971 by Meites and Rogols (4) and additional contributions have been made since then. Even though valuable advances have been achieved in separating the various isoamylases, a simply performed and clinically applicable test for pancreatic isoamylase activity in the serum or urine is still not at hand. Such a test would clearly lend an element of precision presently lacking and serve to enhance the clinical usefulness of this widely employed laboratory test.

Over the past decade or more, a systematic series of studies has been made in our laboratory aimed at the construction of a simple and rapidly performed serum amylase assay method that would reliably identify and measure the amylase activity specifically contributed by the pancreas. This goal has proved elusive and has still not been fully attained. There has been measurable progress, however, with some serendipitous disclosure of clinical value. This presentation is primarily concerned with these studies. In essence it represents a recounting of our experiences to date in an adventure that still continues.

From the Division of Gastroenterology, Department of Medicine, University of California, Irvine, Ca. 92664. Supported by a grant from the John A. Hartford Foundation, Inc.

* Presented as the Ralph Colp Lecture, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029 on April 17, 1975.

Requests for reprints should be sent to: J. Edward Berk, M.D., Department of Medicine, Orange County Medical Center, Orange, Ca. 92668.

Methodology

Electrophoresis. It was apparent to us at the very outset that study of the amylase activity in serum and tissues necessitated the development of reliable procedures for analyzing this enzyme and accurately quantitating its activity. In our initial studies, we employed both an iodometric technique (5) and an adaptation of a saccharogenic method. The saccharogenic method, which was based on a modification of the dinitrosalicyclic acid reaction for measuring reducing sugar, was found to be both sensitive and reproducible (6).

Quantitation of the electrophoretic and chromatographic distribution of human serum amylase, using the modified dinitrosalicyclic acid method, suggested that a majority of the saccharogenic activity in normal human serum, as well as in certain abnormal specimens, exhibited an electrophoretic mobility similar to γ-globulin (6). Since these observations were not consonant with others previously reported using an iodometric method (7, 8), a comparative study of the two methods was undertaken. From this it became clear that there was an intrinsic disparity between the amyloclastic and saccharogenic estimations of serum amylase activity (9).

Studies made on the interaction of human serum proteins with the starch-iodine complex showed that native human serum albumin and γ-globulin interacted with available iodine and were in themselves capable of decolorizing starch-iodine solution to varying degrees (10). This indicated that amylase measurements based on iodometric techniques could yield misleading results and probably accounted for the previous reported findings by others that amylase activity in normal serum resided principally in the same electrophoretic position as albumin.

Previous workers, using an iodometric method for measuring serum amylase, postulated that an amylase inhibitor exists in normal human serum, but not in the sera of patients with acute pancreatitis (8). The principal evidence for this stemmed from the fact that the average amylase activity of normal serum markedly increased after the specimens were subjected to filter paper electrophoresis. By contrast, electrophoretically induced enhancement in amylase activity was not demonstrable in sera from patients with acute pancreatitis. Using our saccharogenic method to measure serum amylase activity, we conducted studies on the sensitivity of this enzyme to heat (11). These observations were made both on sera pooled from normal subjects and on sera obtained from patients with acute pancreatitis. The amylase in the sera of the patients with acute pancreatitis was found to be more susceptible to heat, suggesting that the serum amylase in this condition was of a different molecular form from the normal. However, no evidence was found to indicate that normal human serum contains an electrophoretically labile or separable inhibitor of amylase (11).

Animal Studies

1. *Normal rabbit serum* was found to exhibit amylolytic activity primarily in the γ-globulin zone on paper electrophoretic strips (12). When examined by

DEAE-cellulose column chromatography after prior treatment of the rabbit serum with Sephadex G-25, three major peaks of amylase activity were recorded (13). The material associated with each peak differed in composition of serum protein, electrophoretic distribution of amylolytic activity, optimum pH for activity, and Michaelis-Menten constants for starch hydrolysis. These data were interpreted as suggesting that normal rabbit serum contains at least three molecular forms of amylase, all of which occupy the electrophoretic zone of γ-globulin.

2. Certain properties and enzyme kinetics of electrophoretic and chromatographic fractions of serum amylase were also investigated in *normal and pancreatectomized dogs* (14–16). Two and possibly three amylolytic fractions with different mobilities were distinguished in normal dog serum by paper electrophoresis, and two distinct peaks of activity were demonstrated by Sephadex chromatography. That the fractions represented molecularly different forms of amylase was suggested by dissimilarities in their physicochemical behavior. Moreover, the saccharogenic and amylolytic activity in the γ-globulin zone markedly diminished after pancreatectomy, indicating that the serum amylase fraction appearing in this electrophoretic area in the dog arises in part at least from the pancreas.

3. To place amylolytic activity in the serum of man in better perspective, the amylase activities in the sera of a *variety of mammalian species* were evaluated electrophoretically (15). Distinct variations were found which permitted separation of the mammals into groups. These patterns would appear to be of some significance with respect to the evolution of the isoenzyme pattern of serum amylase in man.

Human Studies

1. *Serum*. Extensive electrophoretic and chromatographic studies of the behavior of human serum amylase established that the majority, if not all, of the saccharogenic activity in normal man and in patients with a variety of pancreatic and other disorders resides in the electrophoretic zone corresponding to γ-globulin (6, 16). The one notable difference that we found in the γ-globulin zone amylase in the various human specimens that we examined was the greater sensitivity to heat of the amylase in the serum of patients with acute pancreatitis (11). In this context, the results of Stiefel and Keller (17) are of interest. They found pancreatic amylase was more labile than the parotid enzyme at temperature extremes. However, on the basis of temperature-sensitivity tests, Warshaw (18) reached the puzzling conclusion that normally circulating amylase in man is neither of pancreatic nor salivary origin. This is at variance with our finding that the pancreatic-type isoamylase was no longer detectable in the serum or urine of patients who had undergone total pancreatectomy (19).

Faced with the need to assess amylolytic activity in very small volumes of eluate, a microsaccharogenic technique was devised (20). Using this method, it was demonstrated that, contrary to prevailing opinion, the serum of the newborn contains reduced, but distinctly measurable, quantities of amylase (21).

2. *Tissue Extracts.* Purified material obtained from human parotid gland and pancreas displayed distinctly different electrophoretic behavior on polyacrylamide gel (22). This not only substantiated the existence of isoamylases in human serum, but also suggested a means for distinguishing amylases of different tissue origins in the circulating blood.

Chromatography

Chromatographic procedures were developed for isolating amylase from human serum and tissues in a highly purified state and free of extraneous protein. This was accomplished through the use of columns packed with various types of a cross-linked dextran gel (Sephadex). The purified nature of the amylase obtained by these procedures was further demonstrated by means of immunodiffusion studies which indicated that the amylase-containing fractions were free of detectable serum proteins.

As already noted, polyacrylamide gel electrophoresis of amylase fractions obtained after dextran gel filtration permitted parotid gland amylase to be distinguished from pancreatic amylase (22). Similar and even more distinct separation was later obtained through the use of a modified chromatographic approach (19). Concentrated serum amylase, partially purified by passage through a column of Sephadex G100, was next passed successively through Sephadex G-25 and DEAE-Sephadex A-50. Material so treated was found to contain two peaks of amylase activity that corresponded in their respective positions with those of pancreatic and salivary amylase (Figs. 1, 2). Normal human urine likewise yielded two peaks of amylase activity after similar treatment, the major component corresponding in its position with that of pancreatic amylase. In serum and urine from patients with acute pancreatitis, the main amylase component corresponded in position to that of pancreatic amylase.

Pancreatic (P-type) isoamylase was found to be absent in sera obtained from 3 of 4 patients in whom total pancreatectomy was supposedly done (Fig. 2) (19). In one of the 4, and in another patient on whom partial pancreatectomy had been done, P-type isoamylase was still present but diminished in amount. It would seem, therefore, that the P-type isoamylase in man indeed represents pancreatic amylase and is not secreted into the blood from other tissue sources of amylase.* The specificity of the isoamylase resembling salivary isoamylase in its chromatographic mobility, however, remains to be established. That it arises from the salivary glands is clear, but it may also take origin from other tissues not yet analyzed.

Limited observations to date on amylases extracted from human liver and from human Fallopian tubes have not revealed any isoamylases peculiar to these tissues. Moreover, the levels found suggest that these tissues contribute little to total serum amylase (19).

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* Observations made in our laboratory subsequent to this presentation indicated that human pancreas also contains a very small amount of S-type isoamylase (Gut 16:1006, 1975).

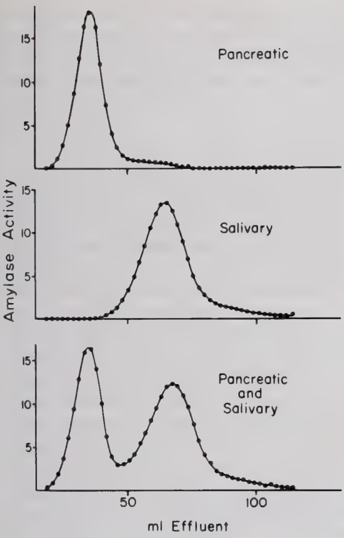

FIG. 1. Separation of partially purified pancreatic and salivary amylase by ion exchange chromatography on DEAE-Sephadex A-50 (Clin Chem 18:1493, 1972; Reproduced with permission of the publishers).

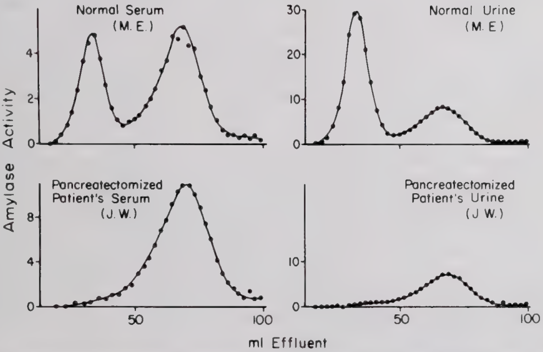

FIG. 2. Comparative isoamylase patterns in a normal subject and a patient who had undergone total pancreatectomy (Clin Chem 18:1493, 1972; Reproduced with permission of the publishers).

More recent experiments have indicated that substitution of QAE-Sephadex A-50 for DEAE-Sephadex A-50 significantly improves separation of the 2 major isoamylases. We have, therefore, been using AQE-Sephadex A-50 in our continuing studies. Reports have appeared from other investigators describing different approaches toward the separation of pancreatic and salivary amylases. Among these is isoelectric focusing (23). The data we have obtained, however, would make it appear that the chromatographic methods we are employing to separate the principal isoamylases are quite adequate and reliable.

Modified Amylase Assay Methods

1. *Automated method.* Since our investigations depended on the location of amylase after paper electrophoresis, polyacrylamide gel electrophoresis, gel filtration, or ultracentrifugation and required examination of multiple samples, we sought to devise a reliable, quantitative, automated saccharogenic method for measuring amylase activity. The method that was ultimately constructed employs the AutoAnalyzer Basic System (24). It substantially overcomes the limitation imposed by the necessity of carrying out saccharogenic assays by a manual method and is applicable to biologic fluids as well as gel column effluents, extracts of paper electropherograms, and similar material.

2. *Use of Chromogenic Substrates.* Attempts were made by us to devise and perfect still other techniques for amylase assay that would permit screening of multiple samples and which were at the same time reliable and simple. To achieve these methods, we turned to the use of chromogenic substrates.

a. *Simplified dye method.* Observations were made on a method using a dye-labeled amylose substrate (Cibachron Blue F_3GA-Amylose). The results correlated well with those obtained with the DNSA saccharogenic method that we had originally constructed (6). To test the applicability of the dye-labeled substrate, observations were made of the hydrolytic effect on it of human serum and saliva, and hog pancreatic α-amylase (25). Maltose markedly inhibited hydrolysis while sucrose did not. Rabbit liver glycogen also inhibited hydrolysis. These findings were interpreted to indicate that hydrolysis of Cibachron Blue F_3GA-Amylose truly reflects amylase activity.

The behaviors of pancreatic and salivary amylases, as well as macroamylasemic serum, were found not to be identical. This suggested that the dye-labeled substrate might have additional potential value as a means of differentiating amylases of different origins and types (26).

b. *Paper spot-test for pronounced hyperamylasemia.* A simple filter paper spot-test was devised to allow rapid screening of samples for pronounced hyperamylasemia. Paper strips (Whatman No. 541) were impregnated with a dye moiety, Ramzol Brilliant Blue R, covalently bound to amylopectin (Amylopectin Azure). Decolorization of the blue paper strip after application of serum or other biological material for 3 minutes indicated that hydrolysis had occurred at the point of application. Observations made on serum samples with differing levels of amylase activity demonstrated that decolorization became evident when amylase activity was in excess of 200 Somogyi units and was clearly apparent when the activity exceeded 500 units (27).

The principle on which this test is based, and its simplicity, make it potentially applicable for wide use in clinical practice. A test of this type would be ideally suited, for example, for use in Emergency Rooms, on the wards of hospitals, and even in physicians' offices. As constructed, however, the test is crude and requires refinement before it can be widely and reliably applied clinically.

Macroamylasemia

In 1967, we published observations on three patients with a disorder that we called "macroamylasemia" (28). This disorder is characterized by pronounced and prolonged hyperamylasemia due to the presence in the blood of a circulating macromolecule of amylase whose large size precludes passage into the urine (Fig. 3). More extended studies made on additional cases formed the basis for later reports on the clinical and laboratory features of this aberration (29, 30). Observations on the nature, composition, and behavioral characteristics of the macroamylase complex were also made (26, 30–35).

In summary, our observations indicated that the macroamylase complex that is the feature of macroamylasemia is not the same in each case. All macroamylase complexes studied by us by means of paper electrophoresis behaved similarly; all showed a single peak of activity between the α-and γ-globulin zones. However, differences were observed in other ways: 1) the complexes displayed differing mobilities through a chromatographic column, such as Sephadex G-200; 2) differences were exhibited in susceptibility to dissociation of the complex under acid conditions; and 3) differences were noted in the ultracentrifugal characteristics of the undissociated complexes. There appeared to be some correlation between ultracentrifugal behavior and

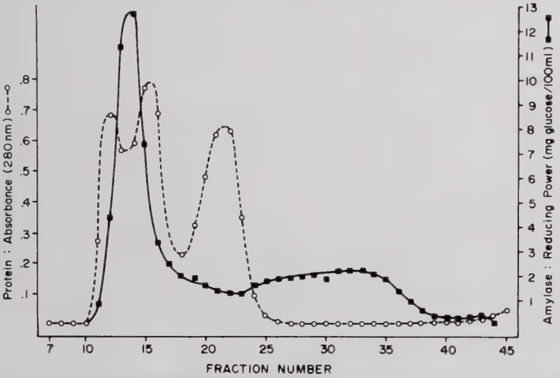

FIG. 3. Elution patterns of normal serum proteins, macroamylase, and normal serum amylase after passage through a microcolumn (1.2 × 11.4 cm) of Sephadex G-200 superfine (Clin Chem 17:423, 1971; Reproduced with permission of the publisher).

susceptibility to dissociation in an acid medium. Those complexes displaying the highest sedimentation coefficients in the ultracentrifuge tended not to be dissociated; those with the lowest coefficients tended to be completely dissociated; those with intermediate coefficients tended to be partially susceptible.

The enzymatically active portion of the macroamylase molecule, when separated by acidification from the macroamylase complex, was found to be similar to normal serum amylase with respect to enzymological properties. On isoamylase analysis, however, a greater proportion was of the S-type (36) and the binding substance in some sera was found to complex only with S-type (37). (These data will be discussed further and in greater detail in the later-appearing discussion of the usefulness of isoamylase analysis.)

We have had the opportunity to date to study 53 patients with hyperamylasemia and demonstrable macroamylasemia. The fact that these cases were seen over a period of some 8 years makes it seem likely that the disorder is more prevalent than originally suspected. To establish better the prevalence of this peculiar aberration, however, we constructed a rapid and simple method for detecting macroamylasemia that could be used to screen large populations (38).

Screening Method. A mixture of serum to be tested, Blue Dextran and cytochrome c is filtered through a small column of Sephadex G-100. Amylase in the effluent fractions is detected by incubation with amylose and subsequent addition of iodine. Macroamylase, if present, is found in the fractions containing the heavier Blue Dextran, while amylase of normal weight appears in the fractions colored by the lighter cytochrome c.

Prevalence. An initial survey using the rapid detection method disclosed a surprising frequency of macroamylasemia in hospitalized patients (39). A subsequent survey using the same screening method confirmed and extended the initial observations (40). Combining the 440 hospitalized patients in the first survey with the 492 hospitalized patients and the 120 diabetic patients in the second series yielded a total of 16 cases of macroamylasemia in 1052 patients (approximately 1.5%). Interestingly, 15 of the 16 macroamylasemic subjects were men. Further, of the 8 patients in the more recent study who were found to have a circulating macroamylase, 5 had total serum amylase activity well within the normal range for the assay method employed. Serial observations made in some patients additionally demonstrated that the macroamylase complex did not persist. The suggestion emanating from these studies is that macroamylasemia may simply represent a phenomenon of binding of normal serum anylase by some protein that appears during a variety of disease states and may then disappear with improvement in the clinical situation.

Clinical Application of Amylase Pattern Analysis

The techniques used by us for differentiating the P- and S-types of isoamylases in serum and urine and other biological fluids have been employed in a variety of clinical situations. The data obtained not only shed light on the amylase alterations to be found in these disease states, but also indicated that this method of analysis was of clinical value in confirming or excluding the existence of pancreatic disease.

1. *Postoperative Hyperamylasemia*

Serum amylase changes occurring in 110 patients undergoing various intra- and extraabdominal operations were serially recorded and isoamylase analysis made in those cases showing a defined significant rise in the postoperative periods (41). Significantly increased total serum amylase was found in 11 (10%) of the 100 patients; in 4 (14%) of those with intraabdominal; and in 7 (9%) of those with extraabdominal operative procedures. The amylase rise was chiefly of the S-type in one of the 4 patients with intraabdominal and in 4 of the 7 patients with extraabdominal procedures. These data demonstrate that postoperative hyperamylasemia occurs surprisingly often and that serum amylase activity may rise even when the surgical procedure is extraabdominal. Moreover, they provide evidence that elevation of serum amylase activity after surgery does not necessarily reflect the existence of pancreatitis and may instead be due to a rise in S-type isoamylase.

2. *Tumor-Associated Hyperamylasemia*

Detailed studies were made of the amylase levels and characteristics in the serum, urine and tumor tissue of 2 patients with metastatic cancer of the lung associated with hyperamylasemia (42). The findings indicated that some cancers of the lung are capable of forming amylase de novo and may even produce this enzyme in prodigious amounts (Fig. 4). Of additional interest was the demonstration in one patient that the amylase formed in the lung tumor tissue was of the S-type. Harada and Kitamura (43) in Japan had earlier described a case with similar findings and Ueda et al (44) have just recorded still another instance.

A series of parallel studies was made by us more recently on the serum and urine of a patient with autopsy-proven primary pancreatic cancer who also displayed pronounced hyperamylasemia and hyperamylasuria (45, 45a). No detectable P-type isoamylase was found in the serum of this patient and only a barely detectable amount of P-type isoamylase was found in the urine using the technique for isoamylase analysis that we have been employing (19). However, when the early fractions recovered from the DEAE-Sephadex A-50 column were rechromatographed, a minute amount of P-type isoamylase could be demonstrated in the serum. The predominant isoamylase form, very surprisingly, was the S-type. An analogous situation has been described by Ono et al (46) in a patient with metastatic cancer arising in aberrant pancreatic tissue in the ileum.

These observations raise many interesting and provocative questions, particularly regarding the behavior of primitive and neoplastic cells. They also

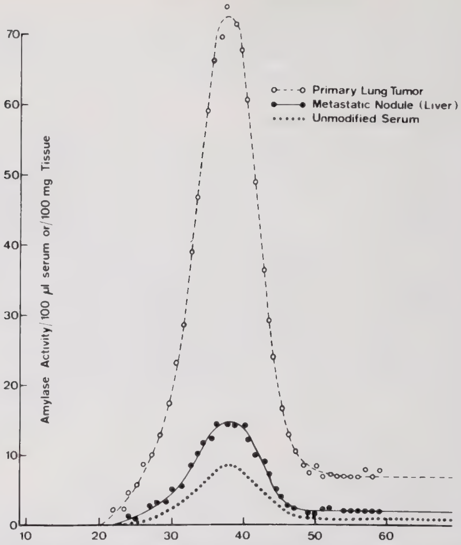

FIG. 4. DEAE-Sephadex chromatographic isoamylase pattern and comparative amylase activity of extracts of the primary lung tumor, a metastatic nodule in the liver and whole unmodified serum of a patient with hyperamylasemia and an anaplastic, round-cell carcinoma of the lung. The single elution peak corresponds in position with that of salivary amylase (Ann Int Med 78:521, 1973; Reproduced with permission of the publishers).

establish an unexpected explanation for the hyperamylasemia that may be presented by some patients with cancer.

3. *Salivary ("S")-Type Hyperamylasemia*

Studies have been made to date in 6 patients with unexplained hyperamylasemia simulating macroamylasemia. The latter disorder was suspected in all these patients because of the finding in them of high levels of serum amylase, low levels of urinary amylase, and low amylase:creatinine clearance ratios. In none of them, however, could a macroamylase be demonstrated in the serum. Isoamylase analysis indicated that there was a preponderance of S-type isoamylase in each case (47). Observations made by us (19) and by others (48, 49) have indicated that S-type isoamylase is less well filtered through the kidney than the P-type, but the precise cause for the elevated S-type isoamylase in these cases is presently obscure. The clinical importance of the disorder, in our present state of understanding of it, rests principally on its simulation of macroamylasemia and the fact that it represents still another cause for persistent hyperamylasemia.

4. *Isoamylase Composition of the Macroamylase Complex in Macroamylasemia*

It was noted earlier that previous studies done by us had indicated a remarkable heterogeneity among the macroamylases. A total of 30 cases of macroamylasemia with complexes susceptible to dissociation at pH 3.4 were studied with respect to the isoamylase nature of the acid-released amylase (36). A much greater degree of variation was found in the isoamylase patterns of the acid-released amylase than was observed in 27 normal sera. This raised the question as to whether the variation reflected variation in the proportion of unbound P- or S-type isoamylase freely circulating in the macroamylasemia serum, or whether they reflected instead some specific properties of the binding substances in the macroamylasemic serum. To answer this, experiments were carried out testing the binding substances (37). Most of the latter were found to display greater affinity for S- than for P-type isoamylase. In one patient, the binding substance bound only salivary amylase and failed to bind detectable amounts of pancreatic amylase. This evidence, suggesting that the variation in isoamylase patterns of the acid-released amylase was due to specific properties of the binding substances in the complex, was further supported by urinary isoamylase analysis in the cases of macroamylasemia.

5. *Identification of Pancreatic Involvement in Clinical Situations*

Isoamylase analyses have been made in series of patients with clinical problems in which pancreatic disorder was a possibility that could not be clearly established or excluded. Determination of the relative amounts of P- and S-types of isoamylase in the serum and urine served in these cases to confirm or exclude the existence of pancreatic involvement with much greater certainty and was of distinct clinical help (50).

Among the cases studied was a patient with diabetic ketoacidosis and hyperamylasemia. Elevation and fall in the level of P-type isoamylase provided evidence for acute pancreatitis during the ketoacidosis; residual hyperamylasemia after subsidence of the acute pancreatitis was shown to be due to concomitant macroamylasemia (51).

More recently, we have conducted studies on another patient referred to us because of abdominal pain, hyperamylasemia, and hyperamylasuria. Tests confirmed that there was a circulating macroamylase. In addition, however, an unusually high proportion of the total serum amylase (approximately 55%) was of normal size and was not bound to any binding substance. This helped explain the unusual finding of the relatively high level of amylase in the urine in the face of macroamylasemia. Studies also revealed that over half of the normal size amylase was P-type, whereas the macroamylase complex, when dissociated, yielded principally S-type isoamylase. It was concluded from these observations that this patient, like the patient with diabetic ketoacidosis referred to above, had concomitant macroamylasemia and acute pancreatic disease. Evidence that the latter existed rested on the demonstration of increased P-type isoamylase in the unbound serum amylase.

Application of isoenzyme analysis in a number of other patients with various disorders proved of help in unraveling puzzling situations by identifying pancreatic involvement. These several observations indicate that isoamylase analysis is a useful addition to the total armamentarium of diagnostic procedures and adds a new dimension to the detection of pancreatic disease.

6. *Amylase in Human Milk*

Use of the modified chromatographic method for isoamylase analysis showed that there was a great deal of amylase activity in human milk (52). It was also found that milk contains S- but no detectable P-type isoamylase. In addition, an "X"-type isoamylase was found to be present which was also present in normal urine and in 3 hyperamylasemic serum samples. These observations indicated that even though lactation theoretically might affect total serum or urine amylase activity, it would not interfere with the detection of pancreatic disease since P-type isoamylase is apparently nonexistent in human milk. If the lactating mammary gland should be shown to synthesize the amylase appearing in the milk, as we suspect is the case, this would be a clear example of nonsalivary origin of S-type isoamylase.

Amylase Changes during Pregnancy

Knowledge of what transpires with respect to serum amylase during pregnancy is of clinical importance because of the occurrence of abdominal symptoms during pregnancy that simulate pancreatitis. Indeed, "pregnancy pancreatitis" has been observed and reported by us (53) as well as by others. However, conflicting observations have been reported regarding the alterations in serum amylase during pregnancy. For this reason, we undertook to study the levels and characteristics of serum amylase in the three trimesters of pregnancy in a group of 200 pregnant but otherwise normal women (54). The results obtained indicated that there was a progressive rise in serum amylase activity to the 25th week of pregnancy; after this there was a less pronounced but gradual fall. More important, an impressively large number of subjects showed values above the 99% confidence level established by us for the method used by us at that time. In addition, determination of the isoamylase distribution pattern in selected samples of high and low levels of amylase activity in each trimester indicated that there was a preponderance of S-type amylase in the second trimester. These findings are of clinical importance because they provide a base for comparison and hence sounder interpretation of the significance of serum amylase values in pregnant women with abdominal symptoms suggesting complicating pancreatitis.

Amylase: Creatinine Clearance Ratio

Reports have appeared in the literature verifying the values of amylase: creatinine clearance ratio as a measure for the ready identification of acute pancreatitis (55–57). The data presented by these reports indicate that this ratio is markedly elevated in the presence of acute pancreatitis and that the elevation exceeds that found in other disorders associated with hyperamylasemia.

We conducted a series of studies in a group of patients without obvious pancreatic disease, but in whom hyperglucagonemia was believed to occur (58). Acting on the hypothesis that hyperglucagonemia in some way enhances renal clearance of amylase, the amylase:creatinine clearance ratios were determined in these subjects. Among the conditions selected for study were diabetic ketoacidosis and burns. The results indicated that patients with these conditions do indeed show elevated amylase:creatinine clearance ratios. It would follow from this, therefore, that elevation of this ratio, while of value in identifying acute pancreatitis, is not specific for nor confined to that disorder.

Studies Currently in Progress

1. *Isoamylase Forms in Cancer*

Passage of serum through Sephadex G-75 rather than G-100 has been found to permit the discernment of other human isoamylases in addition to the basic P- and S-types. Among the additional types is one of unknown origin which we have designated as "X"-type. As noted, this type was found by us in samples of human milk, normal urine, and in some hyperamylasemic sera (52).

Two patients with hyperamylasemia associated with cancer (one bronchogenic and the other pancreatic) were studied through use of Sephadex G100 and DEAE-Sephadex A-50 chromatography for isoamylase composition (45, 45a). P-type isoamylase could not be identified in the serum of either patient. When the serum and urine of these patients was passed through Sephadex G-75 rather than G-100 to obtain more detailed resolution of the isoamylases, a peculiar component, designated "Y"-type, was found in both cases. This component comprised 16.6 to 22.4% of the total amylase activity in the samples studied. Special search of normal serum and urine, pancreatitis serum and urine, serum and urine of patients with S-type hyperamylasemia (47), and mumps serum did not exhibit the presence of Y-type isoamylase. This component did appear, however, in some samples of human milk (approximately 6.8% of the total milk amylase activity).

A feature common to cancer tissue and lactating mammary gland is a high rate of protein synthesis. While this invites speculation, we are hesitant at this point to advance any theories regarding the origin and significance of the Y-type amylase component found in the serum and urine of these cancer patients and in some samples of human milk.

2. *Screening Test for Pancreatic Disease*

Using the background of our previous studies on isoamylase analysis, we have set about devising a new method for specifically detecting P-type hyperamylasuria. A short column (10 × 1.25 cm) of QAE-Sephadex A-50 appears effectively to separate P- from S-type isoamylases at room temperature. The amount of amylase activity in the earliest chromatographic fractions with detectable amylase activity is quantitatively compared with standards prepared from normal persons. For this measurement, the Phadebas dye technique (Pharmacia) is employed. Preliminary data now at hand indicate that normal urine may be distinguished from urine obtained from patients with acute pancreatitis (59). These studies are being pursued in concentrated fashion in hope that the test may prove clinically applicable.

3. *Studies on the Effect of Opiates on Amylase Activity*

Previous observations from our laboratory (2), as well as from other laboratories, indicated that analgesics may produce a rise in serum amylase activity. Such rises could be clinically misleading because the abdominal pain for which the analgesics are given may be erroneously interpreted as due to acute pancreatitis. The mechanism thought to be operative in the rise in serum amylase activity after analgesics is heightened tone of the duodenum, choledochal sphincter and pancreatic ducts, producing elevated pressure in the pancreatic ducts and interference with the flow of pancreatic secretion. Consequent to these effects, amylase is believed to regurgitate into the serum. Whether this is indeed the responsible mechanism is uncertain. Analysis of the isoamylase pattern obtained after analgesic-induced hyperamylasemia would contribute toward settling the issue and for this reason is being pursued.

Thus far, marked hyperamylasemia has been found in 13 of a series of 94 heroin addicts. Twelve of the 13, however, had complicating acute pulmonary changes characterized as "heroin lung" (60) and the remaining patient had status asthmaticus. Ten of the 12 with so-called heroin lung showed dominant S-type elevation; one showed dominant P-type elevation; and one showed a mixed pattern. The patient with status asthmaticus had dominant S-type elevation.

These observations provide no support for the hypothesis that the pancreas is the source in most cases of the amylase that rises in the serum after opiates and other analgesic drugs. The existence of acute pulmonary changes in those addicts with pronounced hyperamylasemia point to a possible relationship between the pulmonary changes and the hyperamylasemia. Studies are now in progress designed to provide some insight into the possibility of a pulmonary source for amylase and its nature.

4. *Amylase Alterations following Retrograde Endoscopic Pancreatography*

Retrograde cannulation of the pancreatic ducts through a fiberoptic duodenoscope is becoming widely used in clinical practice. It has been observed by numerous endoscopists, however, that one hazard attending this examination is the possible production of pancreatitis. Supportive of this is the fairly frequent occurrence of elevations in serum amylase following the procedure (61). Unanswered, however, is the precise significance of the serum amylase elevations occurring after the procedure. To help settle this question, a study is currently underway in which total serum amylase and the isoamylase pattern are being determined before and following upper gastrointestinal endoscopy. These determinations are being made both with and without retrograde catheterization of the pancreatic duct in order to determine if there is an appreciable difference in those patients whose ducts are catheterized. The data at hand are too limited to permit any conclusions. Of interest, however, are our initial observations that whenever the pancreatic duct was visualized radiographically, any concomitant rise in amylase was of the P-type.

Conclusion

Despite nagging problems, disconcerting frustrations, and inability to comprehend or adequately explain all that has been observed, the search for a specific test for pancreatic amylase continues apace. Even though the isoamylase arising from the pancreas (P-type) may now be distinguished from that found in salivary glands and possibly in other tissues as well (S-type), a clinically applicable method which simply and specifically identifies pancreatic isoamylase alone has still not been formulated. Much remains to be accomplished and even more to be understood. The future, however, seems bright with promise.

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Management of Major Gastrointestinal Hemorrhage*

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Gastrointestinal hemorrhage is the most common emergency which confronts the gastroenterologic internist. It is manifested by hematemesis, and by hematochezia or melena, alone or in combination. On occasion, these symptoms are preceded by those of peripheral vascular collapse: pallor, diaphoresis, syncope or faintness, tachycardia, and hypotension. The shock-state may precipitate serious vascular complications (acute myocardial infarction, cerebrovascular accident), renal failure, and it accelerates deterioration of function of a cirrhotic liver. The source of this disorder is located most commonly in the upper gastrointestinal tract, proximal to the ligament of Treitz, less commonly in the colon, and least commonly in the remainder of the small intestine, and in the liver and pancreas and their ductal systems.

The definition of "major" hemorrhage has been predicated upon the degree of severity of the blood loss, i.e., upon the total quantity required to be replaced, and upon the degree of acuteness of the blood loss, i.e., the amount requiring replacement per unit of time. In general, this implies a fall in the hemoglobin content to a level below 8 G/100cc, the red blood cell count below 3,000,000, the hematocrit below 30%, or that a minimum of a liter of blood is required immediately to correct a state of blood volume deficiency.

It is respectfully submitted that all instances of gastrointestinal hemorrhage should be considered to be major at the time that the patient comes under observation. Except in retrospect, there is no way to know with certainty which bleeding episode is, or will become, major.

Fresh, clotted blood may be passed acutely, even accompanied by symptoms of shock, yet the bleeding may cease abruptly, the loss of blood may not even require replacement. It is most commonly in this group of patients that the error is committed of administering a one-unit transfusion, the implications of which, in relation to the transmission of viral hepatitis, are well-known. On the other hand, hematemesis may be massive; and repeated and heroic efforts may be required to keep abreast of the blood loss. Again, what may appear to be a minor bleeding episode initially, i.e., passage of melanotic stool for several days, may be followed by a sudden gushing of blood from either orifice. Finally, blood loss may be relatively slow but continuous. Even the loss of one drop of blood each five seconds, if continued, eventuates in the total loss of almost three units over a period of one day. In

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such instances, the patient may be severely anemic when he comes under medical observation, and may require daily repeated administration of one or more units of blood in order to maintain his hematocrit at a satisfactory level.

Because of these considerations, we have elected, throughout the years, to apply the same basic type of management to all of our patients who have bleeding from the gastrointestinal tract.

As a general rule, the occurrence of internal bleeding generates a sensation of helplessness and of fear in the affected individual. Except for those who are delirious, intoxicated, psychotic, or comatose, we have found these patients to be extremely cooperative with the efforts directed by the physician to their management.

Patients with gastrointestinal bleeding are treated by the Medical Service in the Intensive Care Unit. Their problem requires a joint medical-surgical management; surgical consultation is obtained at an early stage in the course of their hospitalization.

The major considerations involved in the management of the bleeding patient include:

1. Estimation of the degree of severity of the blood loss; 2. Institution of nonsurgical measures to correct it; 3. Establishment of the nature and location of the bleeding source; 4. Control of bleeding; and 5. Prevention of its recurrence; The first two of these objectives demand immediate action. The degree of severity of the blood loss is manifested clinically by pallor, diaphoresis, tachycardia, and hypotension. While hematemesis, especially when accompanied by hematochezia, is a cardinal symptom of the more major hemorrhages, severe bleeding may occur at times, in the absence of these external manifestations. It is important to keep in mind the fact that a hypertensive individual who bleeds may be in shock and yet may demonstrate a level of blood pressure which is normal for nonhypertensive patients. Another point worth mentioning is that the elderly patient may not respond to a decrease in his circulating blood volume with an increase in his heart rate.

The blood pressure and pulse rate are measured and recorded at half-hourly intervals; and the central venous pressure is monitored when this modality is instituted. A flow-sheet is maintained to indicate the volume of fluid intake and output, and the number of units of blood administered.

Blood is drawn for biochemical testing (SMA-12, SMA-6, prothrombin time, etc.), for complete count, and for typing and cross-matching for transfusion. The blood bank is instructed to maintain a reserve of six units of blood at all times while the emergency exists.

Urinalysis, chest x-ray, and electrocardiogram are obtained.

Intravenous administration of fluids is instituted promptly. When hypotension is present, human serum albumin is infused while the arrival of whole blood is awaited. In other instances, a solution of 5% glucose and saline is given by intravenous drip and to it are added arbitrarily 500 mg of ascorbic acid and 75 mg of vitamin K; saline is omitted from the infusate if hepatic or cardiac disease are present.

Although more exact methods are available for measuring blood volume, in our experience, combined monitoring of the hematocrit level and of the central venous pressure has proved to be a practical and satisfactory guide for blood replacement. The hematocrit is determined at four- to six-hour intervals, the central venous pressure is estimated, as frequently as the necessity requires, by means of a catheter whose tip is positioned in the inferior vena cava. Thus, the type of blood (i.e., whole blood or packed red blood cells) as well as the quantity and the rate of infusion can be readily determined. For example, the patient who is anemic (low hematocrit) may have an excessive blood volume because of hemodilution; an appreciation of the existence of this situation will avoid the precipitation of cardiac failure by further transfusion.

Blood is administered when the hematocrit value is below 30% and is transfused with the degree of speed and in the quantity which is needed to bring it to and maintain it at a level of 30%. This is not a magic number; it has been selected arbitrarily as a level to be striven for. The reason for this is quite obvious. In the presence of blood loss, homeostatic mechanisms are called into play by the body in an effort to maintain an adequate circulating blood volume; these involve hemo-concentration and hemo-dilution. If blood is administered to the bleeding patient in amount sufficient to raise the hematocrit level to the normal value of 45%, subsequent hemo-concentration may succeed in raising it to 60%. On the other hand, if the transfusion efforts are directed to maintaining the level at 30%, the individual, following cessation of bleeding, will correct his circulating blood volume and hematocrit level automatically.

While transfusions with whole bank blood usually are adequate for replacement purposes, special consideration is required in the case of patients with liver disease and in those having clotting abnormalities due to other causes. In such cases, it is usually beneficial to administer fresh whole blood, unit for unit, with bank blood. The freshly drawn blood contains clotting factors which are needed by the patient and which deteriorate in bank blood. Although, per individual unit, fresh blood is more expensive than is bank blood, by promoting clotting it assists to stop bleeding, thereby reducing the total number of transfusions which may be required, effecting an overall saving. Fresh frozen plasma, given in adequate quantity is of great value in shortening a prothrombin time which is increased because of hepatocellular disease. Finally, one must keep in mind the necessity for administering calcium gluconate intravenously to those patients who require more than five units of blood transfusion; the excess of anticoagulant in bank blood may depress the coagulating ability of the patients' blood.

In our experience, the greatest shortcoming in the management of patients bleeding from the gastrointestinal tract is the pervasive and chronic tendency to undertransfusion. This is especially true in the case of those patients who bleed massively. Blood replacement is the backbone of therapy for these individuals. It is distressing in the extreme to be called to see a patient, obviously hypovolemic, with tachycardia, hypotension, returning bright red blood from the nasogastric tube, and who is receiving blood transfusion at a rate of 10 to 20 drops per minute! The vital necessity for aggressive replacement of blood is to be emphasized.

The next step is directed to establishing the location and the nature of the bleeding source.

Simple clinical observation is of some degree of value in suggesting the site of origin of the bleeding. The passage of red blood from the oral or anal orifices especially if clotted, indicates its recent escape from the blood vessels. If, following entrance of blood into the lumen of the gastrointestinal tract, there occurs a delay in its transit to either orifice, the actions of gastric and intestinal secretions, and of bacteria and their products, succeed in altering the red color to magenta, to brown, and to black. Thus, the appearance in the stool of bright-red bloody material usually denotes a colonic or ano-rectal bleeding source; of magenta-colored stool, an ileal source (Meckel's diverticulum); of black, tarry material, an upper gastrointestinal source. However, it is possible for the stool to be red in color when the bleeding source is upper gastrointestinal in location, if the transit time is very rapid. Conversely, it may be tarry in color from a colonic bleeding source if its passage is delayed. The discoloration of blood in the vomitus from "coffee-grounds" to black derives from the alteration of hemoglobin to acid-hematin by the action of the gastric secretions.

The occurrence of hematemesis implies a location of the bleeding source proximal to the ligament of Treitz. However, in our experience, hematemesis is lacking in approximately 23% of patients who are bleeding from a source which is located in the upper gastrointestinal tract, these patients demonstrating only melena. Hematemesis occurred less frequently in patients bleeding from duodenal ulcer than it did in patients who bled from gastric ulcer. In any event, the point bears emphasizing that, prior to embarking upon a search for a bleeding source in the upper gastrointestinal tract, it is imperative to ascertain that hematemesis and melena are not the result of blood which has been swallowed from an oral or posterior nasal source.

A nasogastric tube is introduced into the stomach of the patient and secured to the nose with tape. Irrigation of the stomach with ice-water is commenced to monitor the bleeding. The water, in aliquots of 50 ccm, is instilled through the tube and recovered by suction, using a bulb syringe. A total of 250 cc of ice-water is used each time and the process is repeated at intervals of 15 minutes. During the interval between irrigations, the stomach is aspirated continuously by attaching the nasogastric tube to a suction machine. If the returns are continuously clear of blood, the period of monitoring is continued arbitrarily for a period of four hours when the tube is removed. Such an occurrence implies that the bleeding has ceased, or that the source is located distal to the pylorus, or that the bleeding source is not upper gastrointestinal in origin. If the returns are bloody, the irrigation with ice-water and monitoring is continued until the condition of the patient becomes stabilized sufficiently to permit a more definitive diagnostic procedure to be performed which, hopefully, will succeed in establishing not only the location, but also the nature of the bleeding lesion.

The diagnostic modalities which are available for detecting gastrointestinal bleeding sources include:

1. Radiography

    A. Barium contrast studies
        1. Upper gastrointestinal series
        2. Small bowel series
        3. Barium enema
    B. Selective angiography (celiac, superior mesenteric) (1, 2)

2. Endoscopy (esophagogastroduodenoscopy, colonoscopy) (3)
3. Surgical exploration

Endoscopy during active bleeding is superior to radiography in detecting upper gastrointestinal bleeding sources and is relatively simple to perform. Furthermore the ice-water lavage through a large-bore tube which is accomplished prior to diagnostic instrumentation successfully stops the bleeding in the majority of instances. When compared with the results of barium contrast studies, endoscopy has proved successful when radiography:

a. Fails to demonstrate a potential bleeding source;
b. Demonstrates a potential bleeding source which is actually not the true bleeding source;
c. Demonstrates more than one potential source for bleeding, especially when one is located in the esophagus and the others more distally (stomach, duodenum);
d. Demonstrates a lesion which is spurious (blood clots, surgically created deformities)

The endoscopic appearance of certain bleeding lesions is shown in Figures 1-6. In a recent retrospective study we found that x-ray identified the correct bleeding source in 22% of 250 patients with upper gastrointestinal bleeding, an incorrect one in 26%, and failed to demonstrate a lesion in 52%.

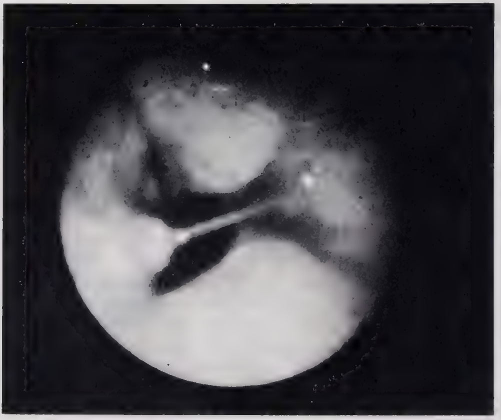

FIG. 1. Bleeding esophageal varix.

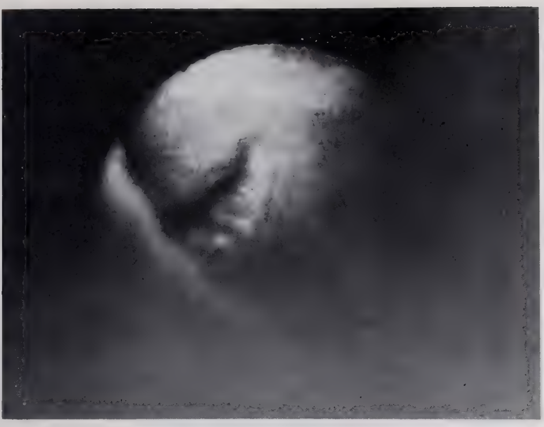

FIG. 2. Mallory-Weiss lesion.

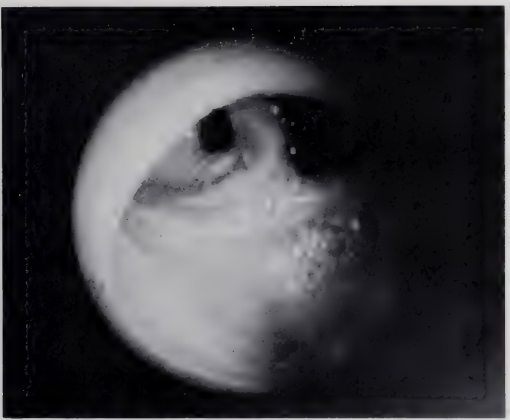

FIG. 3. Large gastric ulcer in antrum filled with fresh blood clot.

Arteriography is an invasive procedure which is not without hazard to the patient. Reactions to the contrast medium which is injected intraarterially, chemical and mechanical damage of the injected vessels, and damage to the femoral artery, occasionally eventuating in a compromise of the circulation and in amputation of the extremity, are factors to be contended with when this

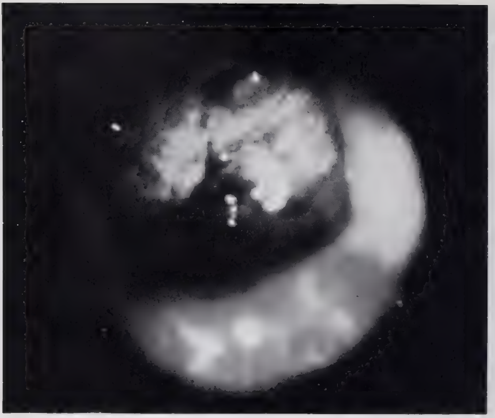

FIG. 4. Large bleeding gastric ulcer.

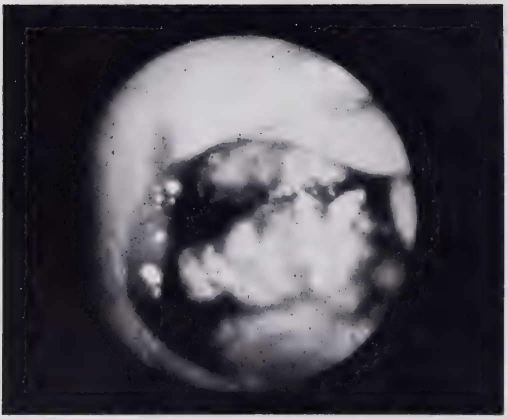

FIG. 5. Bleeding carcinoma of gastric antrum.

procedure is employed. At the present time it probably finds its most helpful application in patients whose bleeding source is located in the small intestine or colon (4).

Endoscopy identifies the bleeding source even after bleeding has ceased. This is in contrast to arteriography which, in order to localize a bleeding

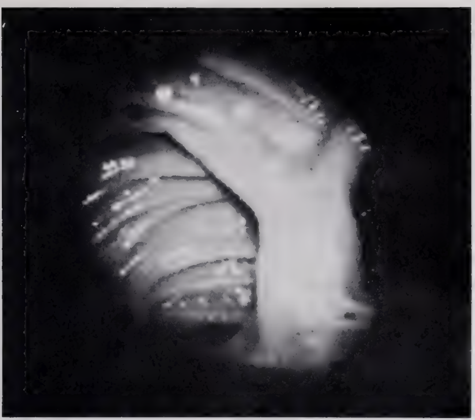

FIG. 6. Bleeding ulcer on spur on BII gastro-jejunostomy.

source, requires that the lesion is actively bleeding at the time that the contrast medium is injected into the pertinent vessel.

Rarely, however, severe bleeding may not be controllable to the degree which is required to permit endoscopic examination. One such instance was observed several months ago in which ice-water lavage of the stomach failed to stanch or to slow the flow of blood from the stomach of a patient whose bleeding was so active and severe that blood was required to be infused in all four extremities in an effort to keep up with the loss. Endoscopic examination succeeded only in exculpating the esophagus as the source. The stomach filled so rapidly with fresh, bright-red blood that visualization was impossible. The patient was rushed to the operating room, rapidly anesthetized, and the abdomen and stomach opened. The bleeding source was found to be a gastric ulcer which had penetrated into the splenic artery.

Successful endoscopic examination of an actively bleeding patient requires that the examiner be skilled in the use of the instruments and have acquired an expertise in the performance of this type of procedure. The number of these individuals is rapidly increasing and the majority of medical centers of importance can claim the availability of one or more of them.

Indications for emergency endoscopy:

A. When the services of a trained endoscopist are readily available
   1. Clinical suspicion of a bleeding lesion in the esophagus, stomach, or duodenum. Also anus, rectum, and colon.
B. When the services of a trained endoscopist are not readily available
   1. Recurrent unexplained bleeding from upper or lower tract
   2. All alcoholics and patients with liver cirrhosis (5)

3. When x-ray examination
    a. Fails to demonstrate a potential bleeding source
    b. Demonstrates more than one potential bleeding source—especially if one is in the esophagus and the other in the stomach or duodenum

C. Prior to surgical exploration for unknown and uncontrollable bleeding

D. Prior to application of esophagogastric balloon tamponade for suspected variceal bleeding

At this point, several pertinent questions demand a reply.

1. Is it necessary, in the management of a bleeding patient, that the location and nature of the source is identified?
2. If so, at which point in time should the diagnostic efforts be made?
3. How accurate is endoscopy in establishing the nature of the bleeding lesion?
4. Do these efforts induce an increased morbidity in the bleeding patient? (Increased bleeding and surgical intervention)
5. Do they have an adverse effect upon the mortality rate of the patient?

The modern approach to the treatment and management of the patient with gastrointestinal bleeding has progressed a long way from the infrequent transfusion and conservative feeding regimen of the 1930's.

A bleeding lesion must be identified and localized accurately because effective therapy is predicated upon this tenet. Many examples can be offered to emphasize this point but the following will suffice.

A patient undergoing treatment for rheumatoid arthritis and receiving steroid medication developed epigastric distress, hematemesis, and melena during the third week of therapy. Upper gastrointestinal radiographic examination demonstrated a deformity of the duodenal bulb. Surgical intervention because of persistent bleeding revealed no ulcer in the duodenum. Gastrotomy was done and fresh blood was seen to enter the stomach from the esophagus. The operation was terminated but the bleeding persisted for several days subsequently. Esophagoscopic examination was done on the second postoperative day which demonstrated the source to be a severe hemorrhagic-erosive esophagitis. If endoscopic examination had been performed initially, surgical intervention would probably have been avoided.

A patient with liver cirrhosis presented with hematemesis and melena. Esophagoscopy demonstrated large esophageal varices which were not bleeding. A bleeding source was demonstrated by gastroscopy in the gastric fundus. However, its nature was not established because the gastroscopic instruments in use at the time lacked the versatility of the modern ones and were incapable of being retroflexed to permit adequate inspection of the fundus region. A diagnosis was made of bleeding lesion in the gastric fundus whose etiology was either ulcer or varices. Surgical operation became necessary because of continued severe bleeding. The gastric fundus was carefully inspected by external palpation and no abnormality was detected. A presumptive diagnosis was made of bleeding gastric varices and a portacaval shunt was performed. The patient continued to bleed following operation and expired. Autopsy disclosed the bleeding source to be a fundal gastric ulcer. The explanation offered by the surgeon regarding his reason for not having opened the stomach for visual inspection of the bleeding site was plausible: he was concerned that the attendant contamination of the peritoneal cavity might induce thrombosis at the site of the veno-venous anastomosis.

A patient with liver cirrhosis developed hematemesis and melena. A recently performed upper gastrointestinal radiographic examination had demonstrated large esophageal varices. It was assumed that these represented the bleeding source and a Sengstaken-Blakemore tube was introduced and inflated in an effort to control bleeding. Unfortunately, the tube was positioned improperly and the gastric balloon was inflated in the esophagus, creating a rupture of the viscus and eventuating in the death of the patient from severe mediastinitis. Autopsy demonstrated a gastric ulcer as bleeding source.

In reply to the second question, endoscopic examination should be accomplished as soon as possible following stabilization of the patient's condition. Of course, clinical judgment must be exercised. In the presence of coma, delirium tremens, acute intoxication, acute myocardial infarction, acute cerebrovascular accident, and terminal states, endoscopic examination should generally be avoided. However, we have had occasion to do endoscopic examinations on several patients who had sustained a recent acute myocardial infarction and who continued to bleed in a life-threatening manner. No harm derived from the procedure and surgical therapy directed to the bleeding lesion demonstrated by endoscopy was successful in stopping the bleeding and contributed to the survival of the patient.

An answer to the question regarding the value of endoscopy, when done during the active bleeding phase, in establishing the nature of the bleeding lesion(s) presupposes that they are located within reach of the instrument. Unfortunately, this fact cannot always be established with certainty. In a recent review of 250 consecutive patients undergoing endoscopic examination because of active bleeding from the upper gastrointestinal tract, the source was established in 89% (Table I).

Regarding the effect of endoscopy upon the morbidity of the patient, it can be stated categorically that, in our experience, we have not noted an increase in bleeding following instrumentation. The ice-water lavage which preceded the endoscopic examination was followed by permanent cessation of bleeding

TABLE I

Endoscopically Established Bleeding Source (250 Patients)

Source	Percent of Patients
Hemorrhagic-Erosive Mucosal Disease (Esophagus and Stomach)	35
Ulcer (Gastric and Duodenal)	33
Varices (Esophagogastric)	12
Mucosal Lacerations (Mallory-Weiss)	7
Neoplasm	2
Source Not Found	11

in most of our patients (58%) and temporary cessation in many others, 28% requiring less than five units of blood transfusion following endoscopy and 14% receiving more than five units. Furthermore, no morbidity occurred which could be ascribed to the instrumentation (Table II).

The number of patients undergoing surgical procedures was not unusual. In actuality, the efforts of the surgeon were directed to the control of bleeding from a lesion whose location and nature he already knew. This undoubtedly reduced the time required for the operation. Twenty-four patients (10%) required emergency surgery because of uncontrolled bleeding. These comprised 6 gastric ulcers, 13 duodenal ulcers, 3 hemorrhagic-erosive gastritis, and 2 varices. As a matter of fact, our surgeons are so satisfied with the assistance provided by the emergency diagnostic endoscopic procedure that they demand that the examination be done prior to performing a surgical operation for control of gastrointestinal bleeding.

Our study lacks a base for comparison between the length of hospitalization of patients undergoing emergency endoscopy and those who did not. However, the period of hospitalization of our patients in relation to the nature of the underlying bleeding lesion and the associated disease states did not appear to be inordinately prolonged.

Finally, no adverse influence on the mortality of the bleeding patient was noted to accrue from the performance of a diagnostic endoscopic procedure during active bleeding. Five (2%) of the patients died from uncontrolled bleeding; eight (3%) died because of postoperative complications.

The statement has been made that "despite enhancement in diagnostic precision [in patients with upper gastrointestinal hemorrhage] mortality rates have remained discouragingly stable at 8% in most reported series" (6). The inference must be decried that "intellectual gratification" is the only value to be derived from the increased diagnostic accuracy provided by the aggressive approach. In evaluating death rates in bleeding patients, several important factors must be considered: age, associated diseases, and especially, the underlying gastrointestinal disorder (7, 8). As noted above, the mortality rate directly attributable to the bleeding lesion was 5.2% in our study. It is submitted, therefore, that instead of advocating a head-in-the-sand attitude as

TABLE II

United Blood Administered/Patient

	Before Endoscopy			After Endoscopy		
	None	( )5	(+)5	None	(−)5	(+)5
Hem.-Erosive Mucosal Disease	42	35	10	57	19	11
Gastric and Duodenal Ulcer	30	39	15	44	26	14
Esophago-Gastric Variances	3	20	7	9	13	8
Mucosal Lacerations (M-W)	4	8	4	10	5	1
Neoplasm	0	5	0	4	0	1
Source Not Found	9	14	5	20	7	1
Total	88	121	41	144	70	36

a consequence of these figures, a reduced mortality may better derive from an improvement in methods for dealing with the gastrointestinal bleeder. We are presently in the process of developing methods for attacking the bleeding source directly by applying electrocautery and laser beams to it through our endoscopes. If these efforts prove to be successful in providing a method for stopping the bleeding, the need for emergency surgical intervention may be eliminated; a definitive surgical procedure, if needed, may then be performed on an elective basis. This may serve to reduce the mortality rate.

Efforts to control bleeding are nonsurgical and surgical. The therapeutic approach which is indicated in any given case varies, and depends upon the nature of the bleeding source. Hence the necessity for the early establishment of an accurate diagnosis.

The gastric lavage with ice-water and utilizing a large-bore rubber tube is successful in practically all instances in removing the intraluminally disseminated blood and clots and in stopping the bleeding. If a variceal bleeding source is established, balloon tamponade is applied immediately following removal of the endoscope, using the Sengstaken-Blakemore tube to which we are very partial. If the condition of the patient permits, failure of the tamponade to control bleeding within four hours, a recurrence of bleeding following release of tamponade 24-hours later, are reasons for portacaval shunt.

In the case of patients with gastric or duodenal ulcer, and those with Mallory-Weiss tears, continued bleeding, those requiring the excess of five units of blood replacement, and bleeding which recurs, are indications for surgical intervention, other things being equal.

When hemorrhagic-erosive lesions of the esophagus, stomach, or duodenum are the bleeding source, a more prolonged effort is made to avoid surgical intervention because of the generally self-limited nature of this disorder. Since the lesions appear to recur in showers and crops, repeated lavage with ice-water through a large-bore rubber tube and with the patient in the head-down prone position, has proved to be of great value in such cases when bleeding recurs. Occasionally, in such cases, intragastric instillation of a thrombin-gelfoam mixture, or of levophed, has proved to be of benefit. At times, direct infusion of pitressin into the arterial vessels that supply the affected segment, delivered through a properly positioned intraarterial catheter, has succeeded in stopping the bleeding.

In those instances where the bleeding source is located in the liver, pancreas, biliary passages, small intestine, colon, or rectum, emergency surgery is indicated for uncontrollable bleeding; if the bleeding ceases, a definitive surgical procedure may be indicated in order to prevent recurrence. The nature of the surgical procedure which is required in any given case is dictated by the nature of the bleeding source.

For those patients with variceal bleeding that ceases with nonsurgical management, elective portacaval shunt following improvement in their liver function offers the best promise for preventing a repetition of the variceal hemorrhage. However, following a successful shunt operation, these patients are equally as vulnerable as anyone else to bleeding from sources other than variceal; furthermore, their life expectancy does not appear to be prolonged because of the operation.

In the case of those patients successfully treated by nonsurgical means for hemorrhage from peptic ulcer disease, a definitive type of surgical procedure should probably be performed if rebleeding from the same type of lesion should occur at some future time.

For those patients bleeding from hemorrhagic-erosive (9) mucosal disease of the stomach and duodenum, and those with multiple gastric ulcers (10) abstinence from the inciting agent (commonly alcohol and/or salycilate-containing medication) usually is adequate to prevent recurrence of bleeding from this source. Abstinence from the use of alcoholic beverages is mandatory for those patients who have bled from varices due to liver cirrhosis and those whose source of bleeding is a Mallory-Weiss laceration.

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STUDENTS' SECTION

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A Review of Neonatal Necrotizing Enterocolitis

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Introduction

Neonatal Necrotizing Enterocolitis (NNE) is a highly lethal disease seen primarily in premature infants. It is characterized by a clinical pattern of gastric retention, bile vomitus, abdominal distention, blood-streaked and occasionally diarrheal stools. It may initially mimic intestinal obstruction, and frequently progresses to intestinal necrosis, perforation, peritonitis, septicemia, shock, rapid deterioration, and death.

The first reported case of NNE was in 1891 by Generisch (1), but it was not until the past few years that the incidence of this disease has significantly increased. Some report an incidence of 3–8% (2) of all premature infants, especially in those who weigh less than 2000 grams, and in an intensive care unit where the pediatricians are alerted to the condition. Unfortunately, in many instances the disease is not diagnosed properly and, unless treated correctly, is lethal in most cases. Overall, the mortality rate remains high, varying from 27–75% (3, 4). Peritonitis and sepsis secondary to intestinal perforation represent the leading causes of death, with intestinal perforation being reported in 33–85% of all deaths from NNE (3).

Clinical Presentation

Clinically, NNE is characterized by temperature instability, lethargy, irritability, prolonged gastric emptying, increased apneic episodes, abdominal distention, and bloody stools in infants weighing less than 2000 grams. Metabolic acidosis often refractory to alkaline therapy, DIC, hypoglycemia, hyponatremia, hypotension, and oliguria also develop in many instances. There is often a history of fetal distress at birth with varying degrees of anoxia following apneic episodes (5). Other reported associated factors have been jaundice, congenital cardiac anomalies, acrocyanosis, twinning, low Apgar (less than six), umbilical catheterization, prenatal fever, prolonged ruptured membranes, infected amniotic contents, exchange transfusions and positive maternal blood, spinal, and stool cultures usually of E. coli, B. proteus, and A. aerogenes (6). These infants, many of whom would have died in the past, are successfully resuscitated and appear well for two to five days. Thereafter, the above signs and symptoms appear. NNE rarely if ever appears at birth, and sometimes manifests itself as late as two months after birth in one reported case (7). Close examination is required to differentiate NNE from Hirschsprung's disease, meconium ileus, intestinal stenosis, or meconium plug

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syndrome. The key to the early diagnosis of this disease is to suspect it, and confirm it on radiographic study. The earliest finding is intestinal dilatation or functional ileus as evidenced by gas-filled distended loops of bowel with air-fluid levels. Pneumatosis intestinalis, or intramural air, is reported present in 75–98% of the infants (2, 4, 7) and is an excellent indication of NNE. It is located most frequently in the colon and appears on x-ray as lines (probably subserosal), cystic spaces (probably submucosal), or both (7). Cystic pneumatosis can occasionally appear as mottled densities resembling fecal matter, but multiple repeat views, or a history of no formula feeding or the presence of linear pneumatosis can usually rule this out. Pneumatosis has also been reported in ischemic gangrene of the bowel, Hirschsprung's disease with associated enterocolitis, and in some forms of bowel obstruction such as small intestinal atresia, pyloric stenosis, and meconium ileus. To differentiate NNE from these entities, the presence of colonic gas and the previous passage of stools make obstruction unlikely (7).

Visualization of gas in the portal tree has also been seen in 33% of the cases (7). It is generally transient, but is considered to be a foreboding sign.

If perforation occurs, pneumoperitoneum may be seen with hyperlucency of the liver area and the outline of the falciform ligament crossing the liver, or it may be confined to the flanks producing a "football" sign (6). Intraperitoneal fluid gives roentgenographic signs of bulging flanks, diffuse abdominal haziness, separation of intestinal loops, and central positioning of floating bowel. These signs can develop rapidly upon perforation with simultaneous disappearance of gas from the bowel wall and portal vein (8).

Pathologic Findings

NNE involves all parts of the gastrointestinal tract, with the ileum, appendix, and colon the most common sites, and the duodenum only rarely. Grossly there is usually dilatation of the involved segments. Moderately involved areas show dullness of the serosa and fibrin deposition. Severely affected sites are discolored with irregular gray to red-black blotches representing hemorrhage and bowel necrosis, usually segmental and circumferential, but occasionally antimesenteric in otherwise normal bowel (9). Bubbles of gas can be seen within the intestinal wall. Often areas where only the serosa is left intact can be seen with its luminal contents (5). Superficial ulceration and submucosal hemorrhage are seen in severely diseased segments with many areas of denuded mucosa covered by a grayish pseudomembrane of agglutinated inflammatory cells, fibrin, and necrotic epithelium (10). Areas of impending perforation in the form of ballooned-out zones or actual perforations are present anywhere, and often the tissue is so friable that handling might fragment it. Gross arterial and venous thrombosis of vessels are absent, as are volvulas and intussusception.

Microscopically, early lesions appear as marked broadening of the mucosal villi secondary to severe dilatation of the venules and capillaries and interstitial edema (5). There is moderate mononuclear cell infiltrate, and occasionally eosinophils are abundant. Some areas of mucosal ulceration are covered with new proliferating epithelium continuous with the surrounding normal epithelium. Some areas of submucosa show granulation tissue with marked fibroplastic proliferation (11).

More advanced stages of disease show hemorrhage in the lamina propria and sloughing of the surface epithelium. Fresh fibrin, debris, and scanty inflammatory cells cover the denuded areas, described before as a pseudomembrane. Frankly necrotic areas are well demarcated with "ghosts" of glands near well-preserved ones (5). Also seen microscopically are subserosal and submucosal gas-filled cysts.

There has been no evidence of vasculitis found, and there is a normal distribution of ganglion cells throughout the intestine.

Associated pathologic findings sometimes include pulmonary atelectasis, intracerebral hemorrhage, petechiae, pulmonary hemorrhage, hyaline membranes, and liver necrosis (12).

Etiology

The cause of NNE remains uncertain. Many theories have been proposed, though most have favored ischemia as the initial inciting event. Some have favored infection as the etiologic factor. However, Roback et al (2) reported that blood and stool cultures did not show a preponderance of any organism, nor was there any seasonal periodicity. Others have suggested a Shwartzman-type reaction related to the formation of endotoxin by gram;negative bacteria acting on a previously sensitized intestinal wall. It is in doubt, however, whether newborns, especially prematures, are capable of such a reaction (12).

Ischemia is held by most investigators to be the most likely cause of NNE, especially following some sort of situation stressful to the newborn, as stated previously. Touloukian and others have advocated that during periods of hypoxia, blood is shunted away from the mesenteric organs to areas of the body that are less resistant to hypoxia, such as the brain and heart. This has been termed the "diving reflex," which has been shown to occur in the duck whenever it dived underwater (10). Roback et al (2) showed similar results in newborn puppies subjected to the stress of posterior hypothalamic stimulation. Touloukian experimentally simulated postnatal respiratory distress, and subsequent resuscitation in piglets. He found that they initially had a reduction and then a rebound above normal in gut perfusion. The piglets then developed focal intestinal hemorrhages, and vascular congestion, presumably the result of the increased capillary fragility produced by the gut ischemia (13–15).

Following diminished blood flow, it can be postulated that the intestinal mucosa undergoes decreased mucin production and proteolytic damage to the gut wall. This, in addition to ishcemic necrosis, causes a break in mucosal integrity, allowing gas-forming organisms to invade the gut wall, forming the pneumatosis intestinalis.

Release of bacterial products causes toxemia as manifested by jaundice, lethargy, and temperature instability. In some cases, the bacteria and their toxins, or gas in the bowel wall, are absorbed into the lymphatics and portal vein, reach the liver, and cause overwhelming septicemia and death. In other instances, necrosis leads to frank perforation, pneumoperitoneum, and generalized peritonitis (10).

Mizrahi et al (16) suggested that intestinal preponderance of gram negative bacteria increased susceptibility to endotoxin. It was theorized that infants fed on cow's milk–based formulas, being deficient in lysozyme, were more susceptible to NNE because their intestinal flora was mostly gram-negative. Roback (2) postulated that oral feeding prior to the onset of the disease may allow NNE to become manifest in a high risk group of infants. The absence of NNE in the first few days of life could be due to the sterile bowel contents at that time. Most infants show stool bacteria only on day three or later, again consistent with the onset of NNE.

Recently Barlow et al (17) reported that most of the infants in their institution who developed NNE had been fed on glucose water or formula prior to the onset of symptoms. These investigators devised an experiment which showed that newborn rats subjected to hypoxia and fed on a formula diet developed an enterocolitis similar to that seen in human infants, while those fed on breast milk and subjected to hypoxia did not. It was also found that breast feeding within the first 24 hours of life and then switching to formula, or instituting breast feeding no later than 24 hours after formula feeding protected the rats from the development of the enterocolitis. Also, any of the rats who received breast milk had no to minimal gram-negative growth in their stool, while the formula-fed rats were heavily colonized with gram-negative bacteria. In fact, rats which were first fed formula and then switched to breast milk showed a rapid and substantial decrease in the number of gram-negative bacteria in their stool.

Barlow et al postulated protection by the breast milk because of its content of IgG, secretory IgA, macrophages, and lymphocytes. Premature human infants, like newborn rats, and unlike full-term infants, are immune deficient, and show a lack of transplacental IgG, delayed secretion of IgA, and depressed uptake and killing by phagocytes. It was hypothesized, therefore, that the ischemically damaged bowel, plus an immature immune system which allowed overgrowth of gram-negative bacteria, results in bacterial invasion into the gut wall, and thence onto NNE.

Medical Management

Successful treatment of NNE depends upon early recognition. NNE must be considered in any newborn that exhibits any of the signs and symptoms mentioned previously. Even before roentgenographic confirmation, treatment should be instituted. All oral feedings are withheld, and nasogastric suction and intravenous fluids are begun. Supplemental oxygen may be given when needed. The infant's blood pressure and urine output should also be followed. Routine x-rays of the abdomen, including supine, AP, upright, and lateral views, should be done immediately, as well as every 4–8 hours to follow the course of the disease and detect a pneumoperitoneum, if it should occur. Barium studies are not usually necessary.

Coagulation studies are monitored closely throughout the course of the disease in expectation of a possible DIC. Systemic antibiotics, such as penicillin, kanamycin, ampicillin, or gentamycin (2, 3, 5), are instituted against enteric bacteria. Positive blood or peritoneal culture and sensitivity studies may necessitate a change of antibiotic. Bell et al (3) advocate the use of topical antibiotics, such as kanamycin or gentamycin, by gavage to prevent perforation, along with systemic antibiotics. They advocate topical treatment to continue for 48 hours after the resolution of radiographic and clinical findings. Nasogastric suction and systemic antibiotics are generally maintained for 7–10 days. Feedings are begun slowly with small volumes of electrolyte-containing solutions, with gradual progression to diluted and then full-strength formulas (2). Attempts to start oral feedings early are often met with exacerbations of their symptoms. For this reason, Roback (2) advocates peripheral and central hyperalimentation during bowel recovery.

Krasna et al (18) reported successful use of Dextran 40 in the prevention of perforation. The major effect of dextran is believed to be due to its ability to decrease platelet adhesiveness. It also coats vascular epithelium, thereby reducing contact factors that initiate coagulation. Dextran also decreases blood viscosity and increases the rate of blood flow. All of these factors presumably play a role in preventing the ischemic processes in the bowel from progressing to complete necrosis and perforation.

Barlow et al (17) have speculated upon the possible use of human breast milk in the premature infant to prevent the development of NNE in the first place. Human milk contains large amounts of IGA, smaller amounts of IgG, active lymphocytes, macrophages, growth enhancers of gram-positive lactobacilli, an anti-staphylococcal agent, complement components, lysozyme, lactoperoxydase and lactoferrin. Any of these might explain colonization of the GI tract in breast-fed infants mainly with L. bifidus, whereas in formula-fed infants, other enteric organisms predominate (E. coli, Clostridium welchii, Streptococci, Bacteroides, etc. (17)). This is similar to that found in newborn rats, who were protected from enterocolitis by breast milk.

Surgical Management

Indications for surgical intervention in NNE include signs of peritonitis, sepsis, perforation as evidenced by pneumoperitoneum on x-ray, sudden clinical deterioration, respiratory insufficiency as evidenced by increasing apnea, hypoxemia, and/or hypercarbia requiring respiratory assistance (9), or clear progression of the disease. Some consider failure to improve within 48 hours of initiation of therapy also a relative indication (4). Other indications include refractory metabolic acidosis, shock, DIC (9), and abscess formation (5). Pneumatosis intestinalis is not considered an indication (10), while there is some disagreement over whether air in the portal tree is an indication for surgical intervention (4, 9, 10).

The operative procedure is obviously dictated by the location, extent, and severity of the involved bowel, and the condition of the infant. Resection is directed at removing all nonviable or acutely inflamed bowel. Stevenson (18) states that primary anastomosis may be desirable and feasible in most localized cases, but exteriorization with ileostomy or colostomy should be performed when extensive resection is necessary, or when the infant is in poor condition. Roback (2), to the contrary, states that primary anastomosis should never be attempted at the time of primary resection. He also suggests the placing of a CVP in the right atrium via a jugular vein at the time of surgery; this CVP to be used for hyperalimentation postoperatively.

Complications

Infants who survive NNE with or without surgery face the risk of several intestinal complications developing anywhere from several days to several months after the disease has subsided. Investigators have reported wound dehiscence and fistula formation (enterocutaneous and enterocolic). Koloske (19) reported two cases of disaccharide intolerance manifested by profuse watery diarrhea, and also acid stools positive for reducing substances whenever the infants were fed lactose-containing formulas. He treated these children with parenteral hyperalimentation and slowly advanced to a normal diet as carbohydrate tolerance improved. Other kinds of malabsorption and malnutrition have also been reported. Sepsis from bowel contamination or catheterization is also a danger. Subcutaneous and pericolonic abscesses and postoperative bowel perforations can also occur. Postoperative pulmonary complications including pulmonary hemorrhage, pneumonia, pneumothorax, pulmonary edema and atelectasis have been reported (9). Generalized bleeding disorders is a very dangerous and fatal complication. Other complications include hypovolemia, hyperkalemia, hypokalemia, seizure activity, subarachnoid hemorrhage, and ileostomy prolapse. In 1970, Krasna et al (20) drew attention to the development of small bowel strictures and colonic stenosis following NNE. This was said to be the end result of the process of healing. Though many of the above complications can occur with or without surgery, some have reported a higher incidence in those infants who required operation in the acute phase of the illness (19), perhaps because they had a more severe form of the disease from the outset.

Lloyd et al (21) reported enterocyst formation—a clear mucoid fluid-filled cyst lined by intestinal mucosa. It was postulated that the cyst was the result of superficial damage and healing by resolution with mucosal regeneration. Proximal and distal stricture would then allow mucosal secretions to accumulate, resulting in the enterocyst.

Rabinowitz et al (22) reported the development of benign lymphoid polyposis. These are globular polypoid projections arising from the mucosa on a broad base. They are composed of lobules of lymphoid tissue with germinal centers. They are benign, self-limiting, with no evidence of malignant change. The etiology is suggested to be a reparative or immunologic response to the previous mucosal damage.

Concluding Remarks

The seriousness of NNE is also demonstrated by a reported median hospital stay of 24 days for infants treated medically, and 63 days for those treated surgically (19). Also, a reported median hospital bill in 1973 for those surviving with surgical intervention was $11,462 (19).

Increased awareness, earlier diagnosis, and improved treatment have increased the survival rate of Neonatal Necrotizing Enterocolitis. Roback (2) reported an overall improvement for survival from 37 to 65%; with survival for those who only required medical therapy improving from 47 to 80%; and for those requiring surgery improving from 32 to 43%. Torma (18) improved his surgical survival from 25 to 58%, and his nonsurgical survival from 0 to 94%. While these and other figures are not necessarily exactly comparable, the trends are still valid.

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Beyond the C. G. Child Classification: Preoperative Parameters Predicting Survival in Patients with Portacaval Shunts

MORTON J. KERN, B.A.

The first portacaval shunt was performed in 1945 by Blakemore, Lord, and Whipple (1). In 1964 C. G. Child and J. G. Turcotte established a set of preoperative parameters to predict the survival of patients undergoing portacaval shunt procedures (1). Child based his prediction of survival on five factors: serum bilirubin, serum albumin, ascites, encephalopathy, and state of nutrition. A classification of patients according to these factors is shown in Table I. The attendant operative mortality of the three clinical groups in Table I has remained approximately 0%, 9%, and 53% respectively for all shunted patients (2).

In recent years attempts have been made to discover additional factors which would improve the predictive accuracy of preshunt studies and survival. Factors that have been studied can be divided into four general groups: clinical findings; laboratory values; hemodynamic factors; and histologic factors.

Unfortunately little predictive value has come from correlation of clinical findings or laboratory values. No correlation between patient age (3, 4), incidence of preoperative variceal hemorrhage (3, 5), continued use of alcohol (5, 6), peptic ulcer (5), or diseases adversely affecting longevity (5) and survival has been adequately demonstrated. The presence of hepatomegaly and jaundice fails to show significant correlation with survival (5) as shown in Table II.

The validity of serum bilirubin and serum albumin as prime indicators of shunt survival has been widely tested and shown to be statistically significant (5–8).

Studies of blood ammonia (3), plasma fibrinogen, factors VII and X, and BSP clearance (4) fail to clearly demonstrate predictive value. There is some question as to the validity of the BSP data. Hsu et al (9) and Jackson et al (5) suggest that low BSP values are related to increased survival. Indeed, the BSP clearance is a very sensitive test of liver function, but is subject to wide variation in altered physiologic states. BSP is transported by serum albumin, a factor often low in cirrhotic patients. This fact may adversely affect the accuracy of the BSP determination. In addition, BSP values depend on hepatic cell uptake, storage, conjugation, and excretion of BSP into the biliary system. Alterations in hepatocellular function seen in cirrhosis adversely

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TABLE I

Clinical and Laboratory Classification of Patients with Cirrhosis in Terms of Hepatic Functional Reserve

Group Designation	"A" Minimal	"B" Moderate	"C" Advanced
Serum bilirubin* (mg. %)	Below 2.0	2.0–3.0	Over 3.0
Serum albumin (gm. %)	Over 3.5	3.0–3.5	Under 3.0
Ascites	None	Easily controlled	Poorly controlled
Neurologic disorder	None	Minimal	Advanced, "coma"
Nutrition	Excellent	Good	Poor, "wasting"

* Equivocal in biliary cirrhosis.

TABLE II

Clinical Findings of Jaundice and Hepatomegaly Related to Survival (5)

Degree of Severity	% Survival	
	Jaundice	Hepatomegaly
none	73	67
mild	56	89
moderate	50	47
severe	40	47

affect accurate BSP values. Until the nature of hepatocellular function in altered physiologic states produced by portal hypertension and cirrhosis can be further elucidated, BSP clearance will most likely remain of questionable value in predicting survival in portacaval shunts. Some insight into hepatocellular changes and survival can be obtained from the work of Mikkelsen et al (10) (vide infra).

Correlation of survival with hemodynamic and histologic factors seems to be more precise than clinical classifications. These factors may lead to a new system of classification for predicting survival in portacaval shunt patients.

Hemodynamic factors may be divided into two groups—first, factors relating to portal blood flow, and second, factors determining overall cardiovascular status. Burchell et al (11) finds in a study of 145 postshunt patients that portal blood flow, when divided into three classes (0–250, 251–500, >500 ml/min), does not significantly correlate with survival. Similarly, when pressure differences between hepatic and portal veins (>250, 250–110, <110 mmH_20), maximum perfusion pressure of the portal system, and splenoportographic staging according to Warren et al (12) were studied, no statistically significant correlation with survival could be found. Burchell et al (11) states that there appears to be no justification for patients with large portal flows to be classified as high surgical risks and those with small flows as good candidates for shunt procedures.

Warren et al (12) propose three hepatic hemodynamic stages to which shunt candidates could be assigned to predict postoperative survival. Table III shows the three stages which had average mortality rates of 35%, 32%, and 60% respectively. It was determined that clinical grouping by C. G. Child's classification was more precise than hepatic hemodynamic staging for prediction of survival. In view of studies on factors relating to portal blood flow, it appears that there is little predictive value to justify the expense or effort in obtaining such data (13).

The second set of hemodynamic factors, those determining the status of the overall cardiovascular system, has been shown to be significant in their prediction of survival. Siegal et al (14), using computer techniques to predict prognosis for portal decompression in patients with cirrhosis and portal hypertension, derived a "survival index" calculated from function curves relating vascular tone, oxygen extraction, relative peripheral shunting, and ventricular function. Abnormalities in relative peripheral shunting have grave prognostic significance. Those patients who died following elective surgery showed the greatest degree of abnormality in effective O_2 transport and peripheral resistance, the two factors from which A-V shunting is calculated. Table IV shows the ratio of survivors to nonsurvivors and the relationship to O_2 transport values and total peripheral resistance.

The degree of relative peripheral shunting clearly separates good risks from poor risks. However, with the addition of cardiac index to the survival statistics, a determination of how well patients with severe cirrhosis will tolerate operation can be made more precisely. The survival index relates net cardiovascular function and survival by measuring cardiac output, O_2 consumption, ventricular stroke work, and central venous pressure. Scores are

TABLE III

*Criteria for Hemodynamic Staging**

Criterion	Stages		
	I	II	III
Estimated hepatic blood flow (EHBF)	>16%	12–16%	<12%
Splenic uptake	0 or 1+	1+ or 2+	2+ or 3+
Splenic pulp pressure	<350	350–450	>450
Varices and collateral circulation	0 or 1+	1+ or 2+	2+ or 3+
Diminished flow velocity	0 or 1+	1+ or 2+	2+ or 3+
Wedged hepatic vein pressure (corrected)	<200	200–300	>300
Free portal pressure	<350	350–400	>400
Maximum perfusion pressure	>175	50–175	<50
Hepatic artery to portal vein flow ratio	<0.5	0.5–1.0	>1.0

* All pressures are expressed in mm of saline. EHBF is the percentage of total blood volume perfusing the liver per minute. Other parameters are qualitatively expressed on a 0 to 3+ scale.

TABLE IV

Survival Related to O_2 Transport and Peripheral Resistance (14)

Survived/Died	O_2 Transport Ml/Ml Blood	Total Peripheral Resistence Dynes/Sec/Cm²
7/4	<15	<1000
10/7	<20	<1500
19/1	>20	>1500

assigned to sets of values individually corresponding to survival rates >90%, 50%, <20%. The scores are then analyzed through various algorithms and a survival index value is derived. The two most important variables appear to be the cardiac index and the extent of peripheral shunting (from O_2 transport and total peripheral resistance) (15). The "survival index" algorithms are shown in Figure 1 and a correlation with survival is shown in Table V.

The significance of the survival index is that overall survival can be predicted better than any other currently used index of liver function or dysfunction (15). The survival index permits accurate prediction of high and low operative risks in patients in whom there is no significant difference based on liver function studies. More importantly, survival prediction may be improved by observation of changes in the survival index after the administration of rugs which alter cardiac output (digitalis, isoproterenol, vasodilators, etc.), peripheral resistance, or venous pressure in a manner which increases the computed survival index score.

One of the most promising criteria for predicting postshunt mortality appears to be the histologic appearance of a liver biopsy done prior to operation.

In a study of 145 patients in France by Maillard et al (10), the presence of fibrosis and inflammation was significant with respect to survival up to the end of the first postoperative year only. Mikkelsen and Kern suggest that the presence of moderate and severe degrees of acute hyaline necrosis on biopsy markedly and adversely affect overall mortality in shunt patients (10).

The mortality for all patients with acute hyaline necrosis (AHN) is 65%. Patients having emergency portacaval shunts had an operative mortality of 83% if AHN was present. Patients undergoing shunts without AHN on biopsy had an operative mortality of only 6% (10).

Three series of patients were studied from 1961 to 1972 by Mikkelsen et al in an attempt to correlate AHN and other histologic findings with survival as shown in Table VI. Mikkelsen also found that patients with AHN may fit into any of the C. G. Child classifications (see Table VII). If moderate to severe hyaline necrosis is seen on liver biopsy, surgery cannot be recommended even in a patient whose clinical and laboratory findings suggest that he would be a good operative candidate from within the Child's classification. Although at present evidence is meager, patients in whom hyaline necrosis has resolved will most likely be good surgical risks (10).

Histologic changes in a single liver biopsy appear to be accurate and important prognostic indicators in shunt patients. Moreover, it may be

TABLE V

Survival Related to the "Survival Index" Score (14)

Survival Index Score	Survived/Died (%)
>0.0	17/0 (100)
−0.1 to −50	8/9 (47)
< −50	2/8 (20)

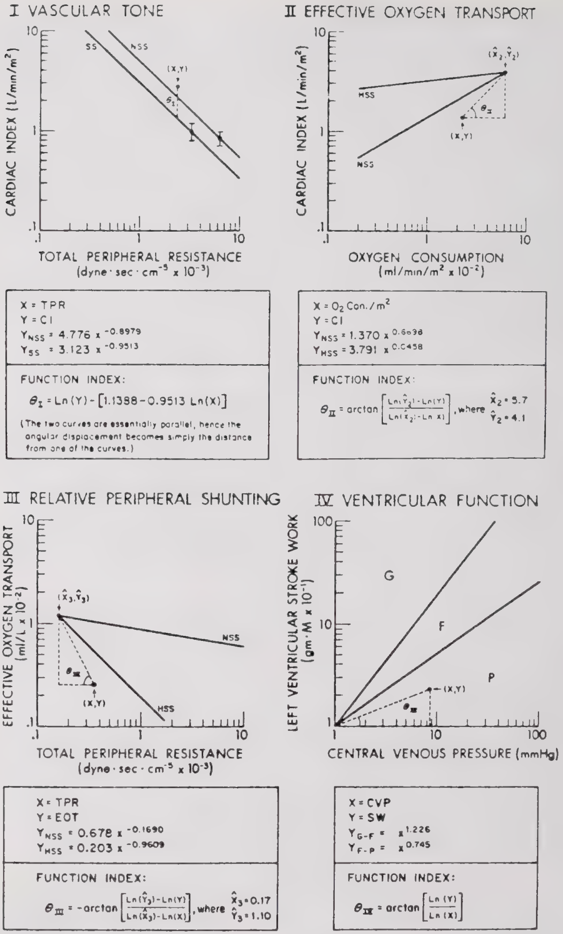


FIG. 1. Determinants of the survival index; display of function curves (Reference 14). Reproduced with permission.

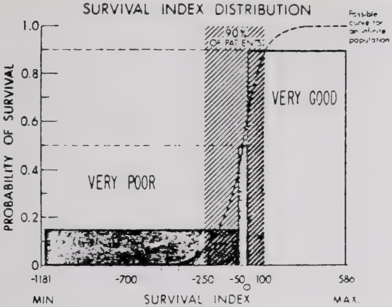


FIG. 2. The Survival Index distribution as a function of the probability of survival (Reference 14). Reproduced with permission.

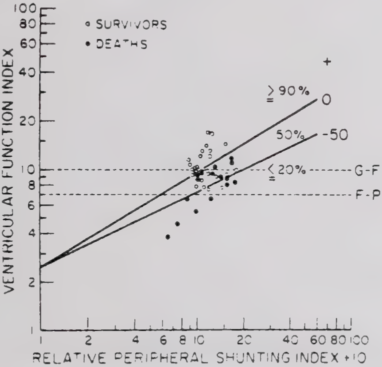


FIG. 3. Survival pattern based on the preoperative determination of the indices of Relative Peripheral Shunting and Ventricular Function in 44 patients with hepatic cirrhosis and portal hypertension prior to portal decompressive surgery (Reference 14). Reproduced with permission.

TABLE VI

Histologic Class and Survival (10)

Degree of Pathologic Changes	Series*		
	I	II	III
0	50/2**	32/1	18/1
1+	7/0	1/0	6/0
2+	4/5	3/3	1/2
3+	5/10	4/10	0/0

* I—1961-1972; II—1961-1967; III—1968-1972.

** Survived/died.

possible with advanced techniques used in the study of hepatic cell function (e.g., electronmicroscopy, immunofluorescent staining, immunoradiographic techniques, etc.) to determine with high accuracy the exact state of the hepatic cell in cirrhosis and portal hypertension, and to predict to what extent the cell will respond to the neophysiologic changes induced by portacaval shunting. By following histologic changes with serial liver biopsy, improved therapeutic management regimens of the postshunt patient may hopefully lead to lowered mortality.

Summary

C. G. Child in 1964 established clinical groups to predict survival in patients with portacaval shunts. Four groups of factors were discussed to evaluate their impact on improving predictive accuracy preoperatively.

TABLE VII

Presence of Acute Hyaline Necrosis and Other Predictive Factors (10)

Factors	AHN present	AHN absent
serum bilirubin	2.5 mg%	1.5 mg%
serum albumin	3.1	3.3
Prothrombin time	61%	66%
Ascites present	60%	35%

TABLE VIII

Prognosis of 150 Patients with Malignant Tumor of the Small Intestine According to Histologic Type of Tumor

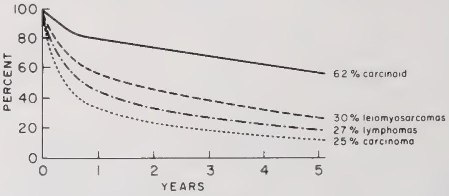
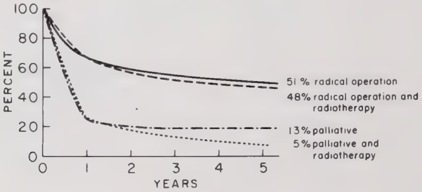


TABLE IX

Patients Having Radical vs. Palliative Surgery, Depending on the Extent of the Lesion Involvement



The use of clinical findings such as age, incidence of preoperative hemorrhage, and laboratory determinations of serum proteins and BSP clearance did not improve on the predictive accuracy of the Child classification.

Of the hemodynamic factors, portal flow determinants and systemic cardiovascular determinants, only the cardiovascular determinants, arranged and computed in a "survival index" by Siegel et al (14), appeared to affect predictions. The survival index appears to be a better predictor of survival than any of the indices of hepatic function or dysfunction.

Correlation of hepatic histologic findings and postoperative survival is of highly accurate predictive significance and may lead to the lowering of operative mortality. With the development of advanced histologic techniques, the state of hepatocellular function could be the best single prognosticator of survival. Until such time, the Child's classification remains the most reliable criteria on which to predict portacaval shunt survival.

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Psychological Problems following Ileostomy and Colostomy

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Resection of colon and rectum resulting in formation of a permanent colostomy or ileostomy is a fairly common procedure and is associated with many psychological problems. Surprisingly, the literature on this topic is scant and found almost exclusively in psychiatric and nursing, rather than surgical, journals. Very little research has been done in the area and most of the information known has been drawn from questionnaires and limited numbers of individual case reports.

The surgeon is usually the person to whom the ostomy patient turns with his problems, especially in the immediate postoperative period. For this reason it is most important that the surgeon be aware of the psychological problems of the patient and be able effectively to help the patient deal with these problems (1). It has even been suggested that the patient's ultimate success or failure with the ostomy may be to a great extent a function of the support and encouragement he receives from doctors and nurses while in the hospital.

Obviously, the patient's psychological functioning prior to the surgery is vitally important in determining his reaction to the ostomy. Equally important is the disease for which the resection and ostomy is performed. In general these patients fall into two groups: the young person with ulcerative colitis with a history of incapacitating disease with its psychogenic component; and the older person with cancer and relatively few symptoms but with the possibility of an awesome prognosis (2). Given the wide differences among these patients, there are general problems and concepts that may be applied to all ostomy patients.

Immediately postoperatively, the patients are almost universally shocked to see the ostomy. In one study even the patients who claimed not to feel this, replied to the question of what they first thought by saying, "I was not horrified" and "It was not disgusting and ugly" (2). The first response is one of denial, of not wanting to look. The denial of the loss of the rectum, of the reality of the stoma, may result in the sensation of "phantom rectum." The patient may experience the sense of rectal fullness and urge to defecate for a few days to a few weeks. Many patients are confused about the anatomy of their "insides" and what exactly was removed and rearranged (3).

The change in body image is a major problem for ostomy patients. The feeling of distortion of self may result in much anxiety and in mourning for the lost part. The ostomy is viewed as mutilation of the abdomen, an unsightly

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From Mount Sinai School of Medicine of The City University of New York, N.Y. 10029, submitted as a mini-thesis for the Department of surgery while Dr. Morrow was a fourth-year medical student. Dr. Morrow received her M.D. degree in June 1976.

orifice. The surgery is often viewed as a physical or sexual assault on the body, a violation of body intactness (3). A study of responses to the Rorschach test by 31 patients with colostomies for cancer showed that most of the patients regarded their body as seriously injured and were overly conscious and concerned with body anatomy (3). In another survey many patients felt that their energy and vitality was very much dependent on certain organs. Upon removal of an important organ, the patient saw himself as fragile and weak. This resulted in a reduction in function and in restriction of roles (3). The revision of how the patient perceived himself and his relationship to others affected his autonomy and ability to function. More than just the loss of an important organ is the problem of the unsightly new orifice. The patient experiences profound feelings of horror, shame, degradation, and fear of rejection by others.

The gastrointestinal tract is the most frequent focus of psychosomatic symptoms. This may be viewed partially as a result of the attention given to it in infancy and childhood. In infancy, feeding and elimination are intimate experiences with the mother figure. Bowel function is learned early and is highly charged emotionally. Children struggle with parents over when, where and how to defecate. Learning control of bowels is a means of gaining love and support from the parents (4). Parents stress the importance of cleanliness; for the child, soiling is risking loss of acceptance and love. Control of the anal sphincter as a child has many psychological ramifications. Loss of the control and reliving of the struggle to regain control of bowel function widely affects self-respect, interpersonal relationships, and other functions. This time the struggle is without parental love and encouragement, which must now be supplied by doctors, family, and friends (5).

The importance of the surgeon's influence should be emphasized. Especially immediately after the surgery, the surgeon is the most important person in the patient's life. The patient will turn to the surgeon with all his problems in a state of extreme dependency after the surgery. The surgeon may be viewed as curer and healer or as violator and mutilator (6, 7). However, the anger at the surgeon for the mutilation has to be repressed because the patient is dependent on the surgeon. The patient is afraid of antagonizing the person he needs the most. As a result, the patient may be afraid of discussing certain of his feelings or problems with the surgeon (2). If the violation or mutilation takes on sexual connotations for the patient, the surgeon may become the object of erotic fantasies and emotional attachment, especially in women patients (8). Acceptance by family, especially spouses, is critically important at home; but while in the hospital, acceptance and encouragement by the surgeon and nursing personnel is vital.

Sexual problems may result in ostomy patients. A physical basis for this is found in men with abdominal-perineal resections with interruption of autonomic nerves resulting in impotence. Added to this are the many emotional problems involving feelings of physical unattractiveness, loss of interest in intimacy, impotence, and frigidity. The stoma itself may take on sexual significance. Some men may view the stoma as a vagina and any bleeding from the stoma as similar to menstruation. They may view the surgery as feminizing, as "being turned into a woman" (2, 3). Women may view the stoma as a second vagina. Case reports suggest that the stoma may also become like a penis for some patients and erotic pleasure may be derived from manipulation of the stoma. Some degree of exhibitionism may develop in these patients (9, 10). Literature dealing with the sexual problems of these patients is exceedingly limited. The questionnaire method of research used in most cases does not lend itself to much insight into this problem.

Many surveys have shown that in general ostomy patients develop a new order of living and a change in personality characterized by conscious control of the range and type of social participation. There is a tendency toward withdrawal and seclusion, perhaps in an attempt to avoid social rejection. There is a tendency toward regression in thought and behavior resulting in restriction of interests and withdrawal of emotional involvements. This is accentuated by centering of schedules and interests around the stoma (5). The patients have been called "prisoners of the bathroom." In one survey, 72% of the patients responded that they had decreased their social relationships. Most often the reason given was fear of odor or spillage (2). Many patients develop elaborate, inflexible routines for care of their ostomies. This is thought to serve as a substitute for control over bowel function. In the Rorschach evaluation of ostomy patients it was concluded that in general the patients seemed to have constricted personalities, with feelings of helplessness, inadequacy and rigidity. Depression is a common result of these feelings and problems (3).

Summary

Psychological sequelae following a colostomy or ileostomy are important and very real problems, although poorly researched and discussed. Even though support of the patient by family, friends and ostomy clubs is essential in his rehabilitation, the central role of the surgeon should not be minimized. Therefore, it is extremely important that the surgeon be familiar with and effective in dealing with these special problems.

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RESIDENTS' SECTION

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Epidermoid Cyst of the Spleen: Case Report and Discussion

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In the absence of infections, hematologic or lymphoproliferative disorders, primary splenic pathology is commonly neglected in the differential diagnosis of abdominal masses. However, the spleen may be the site of origin of a variety of cystic and solid tumors. We report a case of epidermoid cyst of the spleen presenting as a large asymptomatic abdominal mass in a young woman.

Case Report

E. H., a twenty-one-year-old white woman was admitted to the gastroenterologic service at Mount Sinai Hospital because of an abdominal mass found on physical examination in a physician's office. The patient was in excellent health and had sought medical attention because of gas sounds in the stomach. Medical history was otherwise within normal limits.

A large, smooth, nontender, soft mass was palpated in the left upper quadrant of the abdomen. The mass moved only slightly on deep inspiration. There was no other palpable organomegaly. Pelvic and rectal examinations were normal.

Hemoglobin was 14.6 grams %, hematocrit 44.8%, reticulocyte count 1.8%, white blood count 7,200 per cubic mm., and platelet count 185,000/mm^3. Bone marrow aspirate revealed only erythroid hyperplasia with normal iron stores. All other laboratory examinations were normal.

Flat film of the abdomen revealed a noncalcified mass in the left upper quadrant. On intravenous pyelogram, the left kidney was displaced inferiorly and medially (Fig. 1). The ureters were normal with no evidence of obstructive uropathy. The stomach was displaced medially on upper gastrointestinal series (Figure 2). A liver-spleen scan revealed a mass in the area of the spleen displacing the left lobe of the liver to the right, Sonogram of the abdomen revealed a large cystic structure displacing the liver to the right so that it was almost vertical in position against the abdominal wall. The preoperative diagnosis was a cystic mass of the spleen.

At laparotomy, a large splenic cyst was found extending from the diaphragm superiorly to past the brim of the pelvis inferiorly, and also beyond the midline of the abdomen. There was very little splenic tissue surrounding the cyst, which was yellowish gray in color. Cross-section of the cyst revealed the wall to be trabeculated; the fluid inside the cyst was of greenish-black color containing necrotic material. Histologic examination revealed the cyst wall to be composed of squamous epithelium. Final pathologic diagnosis was that of epidermoid cyst of the spleen.

The postoperative course was uneventful and she was discharged on the ninth postoperative day.

Discussion

Cysts of the spleen are an uncommon entity (1). Although two-thirds of all splenic cysts are echinococcal (2), fewer than 600 nonparasitic cases have been reported in the world literature. Although they are more common than solid

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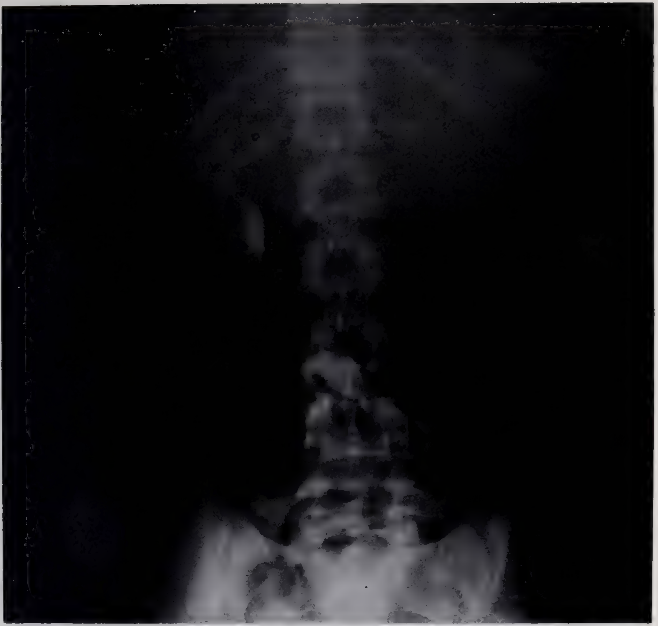

FIG. 1. Intravenous pyelogram showing inferior and medial displacement of the left kidney which is located anterior to the lumbar vertebrae. The right kidney is in normal position.

tumors of the spleen, splenic cysts occur less frequently than their renal, ovarian, and pancreatic counterparts. Splenic pseudocysts are usually secondary to trauma and are four times more common than true splenic cysts (2). Epidermoid cysts comprise about ten percent of all nonparasitic splenic cysts; just over 50 cases have been reported in the literature. Cystic hemangiomas (3) are slightly more common, whereas dermoid cysts (4) are much rarer.

Splenic cysts are conveniently classified as (5, 6):

I. Primary with cellular lining. Component endothelium.
  a. Congenital
    (1) Serous
    (2) Transitional cell-lined (noncomponent epithelium)
  b. Traumatic
  c. Inflammatory
    (1) Infoliation cysts
    (2) Dilation cysts; lymphangiectatic, polycystic disease

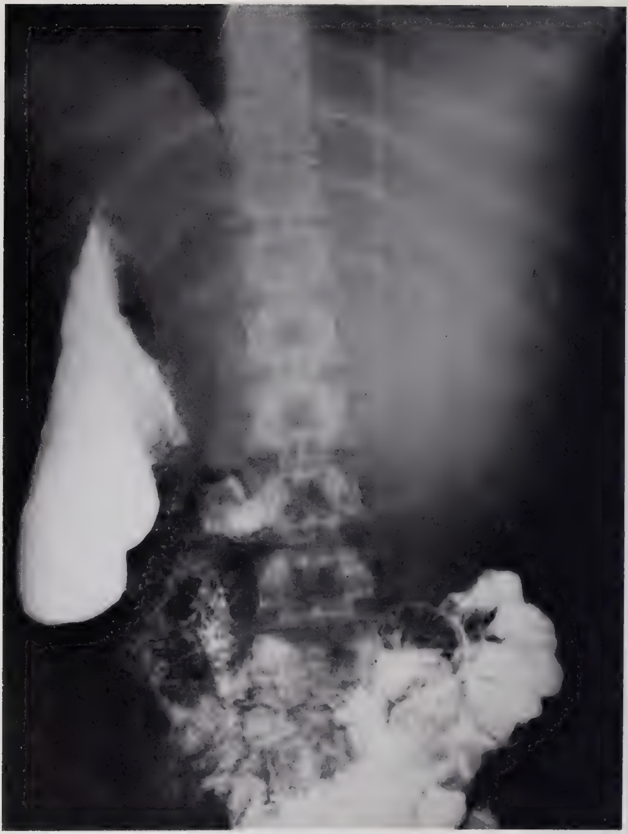

FIG. 2. Upper gastrointestinal series showing displacement of the stomach into the right upper quadrant. Note the homogeneous opacification caused by the cystic mass in the left upper quadrant.

d. Neoplastic cysts (also congenital)
    (1) Epidermoid noncomponent epithelium
    (2) Dermoid noncomponent epithelium
    (3) Lymphangioma
    (4) Benign, cancerous, and capillary hemangioma

II. Secondary, no cellular lining
    a. Traumatic (blood and serous types)
        b. Degenerative (liquefaction)
        c. Inflammatory (necrosis, tuberculosis)

Epidermoid cysts of the spleen were first reported in 1929 in a girl aged nineteen and a boy aged fourteen (7, 8). These cysts are most common in the second, third and fourth decades of life with 80% occurring in patients under twenty years of age. The youngest reported case was in the first year of life and the oldest in a woman in her early fifties (2). Epidermoid splenic cysts occur almost equally in both sexes (9).

Most patients have a history of increasing abdominal girth or with a left-sided abdominal mass. Usually symptoms are otherwise negligible, with pain and abdominal fullness being the most common. Most patients notice increasing abdominal size for an average of a year and a half before seeking medical attention (9). Several patients have acute symptoms caused by infection or by rupture of the cyst (1).

Although the symptoms are usually innocuous, most epidermoid cysts of the spleen nevertheless become symptomatic, requiring medical attention (1). Incidental diagnosis at autopsy is exceeding rare. Physical examination generally reveals a huge mass or tumor filling much of the left side of the abdomen.

Because of the slow rate of growth and trivial nature of the symptoms, epidermoid cysts of the spleen may be very large when diagnosed. Gross splenomegaly in an otherwise normal child or young adult should suggest the diagnosis. All causes of primary and secondary splenomegaly must be ruled out; liver, kidney, ovary, and retroperitoneal pathology must also be excluded.

Radiographic studies invariably show displacement of the adjacent organs by a space-occupying lesion of low density. Chest x-ray may show an elevated left diaphragm. Intravenous pyleogram usually reveals a depressed left kidney, although occasionally the kidney may be elevated. A cyst has been reported to compress the left kidney and excretory system, diminishing renal function (9). Calcification may be present but is rare. Our patient did not evidence calcification. Barium contrast studies show medial displacement of the stomach and downward compression both of small and large intestines. Scanning and arteriography reveal nonvascular, space-occupying lesions. Some authors have suggested that arteriography is contraindicated, because the information gained does not justify the risks involved. Moreover, noninvasive studies yield sufficient information for the diagnosis to be made.

Some authors in this country advocate percutaneous aspiration of the cysts on the grounds that parasitic cysts are rare in this country (2). Others, including ourselves, do not agree because there is an increasing number of immigrants from southern Europe and the Middle East in this country. Aspiration of a hydatid cyst with abdominal contamination would be a catastrophe. Moreover, aspiration of an infected cyst is also potentially hazardous.

Splenectomy is the treatment of choice (9). It is associated with less morbidity than enucleation or marsupialization. When the cyst has already ruptured, splenectomy should still be performed and the abdomen adequately drained. If splenectomy is contraindicated, incision and drainage can be performed only if it ascertained that the cyst is not of parasitic origin. However, this is a most unsatisfactory solution.

Complications of splenic epidermoid cysts are usually related to the size of the cysts. Large cysts are easily traumatized and susceptible to rupture. Several cases of cyst infection have been reported.

Pathologically, epidermoid cysts of the spleen are defined as those cysts which are lined in part with stratified squamous epithelium with intracellular bridges (1). Some challenge this definition, claiming the cells are really metaplastic mesodermal tissues (10). The precise etiology is unknown. Perhaps the ectoderm in the spleen is derived from a primitive totipotential cell capable of producing any of the three layers, such as is postulated to explain the ovarian and testicular dermoids. These cysts are usually large with a dense fibrous lining that may be smooth or trabeculated. Eighty percent are solitary, but satellite cysts may exist either in the cyst wall or elsewhere in the splenic tissue. Most are in the lower pole of the spleen and are subcapsular. They may contain several liters of clear or cloudy fluid with liquefied fat or blood from previous hemorrhage. In addition, the remainder of the spleen may be enlarged because of pressure of the splenic vein, resulting in congestive splenomegaly.

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Hematologic and Immunologic Aspects of Parabiosis*

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Introduction

Parabiosis, the surgical union of two animals allowing the interchange of blood constituents, has been used as a model in metabolic and physiologic research for many years (1). Most experiments were of short duration (2) because histocompatibility differences between non-inbred members of even the same species resulted in reciprocal immunologic reactions by each partner against the other. Furthermore, all immunologic investigations were hampered by this lack of genetic uniformity in animal models. The immunologic heterogeneity resulting from these genetic differences thus made the results of parabiotic union unpredictable even when performed between animals of the same litter.

In the early 1950's, repeated inbreeding and back-crossing of various animal colonies produced a number of viable inbred strains of mice and rats that have uniform or almost identical genetic composition with all traits expressed by homozygous alleles. Long-term survival of syngeneic parabionts of the same strain was routinely possible. Also, syngeneic parabionts could be used as controls for the investigation of the immunologic consequences of parabiosis between parental inbred animals of different strains (allogeneic) and the parabiosis of one parental strain to its F-1 hybrid offspring (semi-allogeneic).

Technique

The technique of parabiosis performed in our laboratory entails the primary union of skin and muscle body wall as well as the creation of a celomic cavity anastomosis (4). An incision is made from behind the ear to the anterior aspect of the rear leg on the right and left side of each partner animal respectively, and the ventral skin edges are joined. Anastomosis between abdominal cavities is then performed. The musculature of the thoracic and abdominal walls are sutured together and the scapulas united. Finally, the dorsal skin edges are joined (Fig. 1).

Postoperatively the parabionts have difficulty in coordinated movement,

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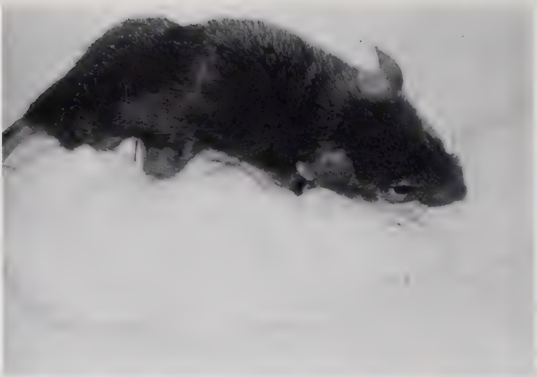

FIG. 1. CBA/J mouse (brown) parabiosed to an A/J mouse (white) immediately after surgery.

especially during eating and in drinking for several days. By the end of the first week, coordinated action is no longer a problem. Weight loss is considerable and may continue for several weeks until a plateau is reached. From this point, the growth curve parallels that of a single animal but the usual adult weight is never attained by either parabiont (Fig. 2) (6).

Parabiotic Intoxication: In both allogeneic and semi-allogeneic models, histocompatibility differences between parabionts cause characteristic immunologic reactions to occur (7–9). These syndromes are grouped under the heading of parabiotic intoxication (10). In allogeneic parabiosis, each animal can react against its partner. In semi-allogeneic parabiosis, the parent animal reacts immunologically against the F-1 hybrid; however, the hybrid cannot react against the parent for the latter has no antigens which are not shared by the hybrid (10). This reaction is equivalent to a graft versus host reaction (GVH) in which the parental animal is the graft and then the hybrid animal is the host (11).

The immunologic consequences of parabiosis differ in some respects from classic models of GVH reactions. GVH reactions between strongly histocompatibile animals when elicited are uniformly fatal unless suppressive therapy is instituted (13). Semi-allogeneic and allogeneic parabiosis are associated with high mortality rates depending upon the strains involved, but not all parabiosis results in death. Also, even in strain combinations in which mortality usually occurs, the parabionts can be saved if they are separated before a critical point in time is reached (12).

Single animals affected by GVH reactions develop a runting syndrome (11), with weight loss, lethargy, hair loss, hepatosplenomegaly, hunched posture and watery diarrhea. Exposed skin areas become erythematous and edematous. Nonsyngeneic parabionts also develop a runted appearance. However, the peripheral edema and erythema are minimal and although the stool softens, diarrhea does not occur. If the parabionts survive, they lose the runted characteristics and appear normal. Splenomegaly develops and then regresses (12).

Healing of the parabiotic union takes place by capillary and connective tissue proliferation in a manner similar to that of skin grafts (10). After the skin external to the cutaneous suture lines sloughs, the skin edges unite and merge with little or no inflammation. Occasionally, there is minor separation of skin edges at the anterior aspect of the dorsal union behind the scapula. However, if these areas are irrigated and resutured, healing ensues even in allogeneic pairs (14). When other measurable parameters indicate that substantial immunologic reactions are occurring, the tissue junctions do not necessarily break down.

The celomic anastomosis remains patent even after as long as eight weeks of union (14). The edges of the body walls involved in the anastomosis remain joined and show scar formation. Occasionally bowel has been noted to herniate through the anastomosis but this is not always associated with intestinal obstruction. In some pairs, the spleen of the right-sided animal has been found intact within the body cavity of the left-sided animal. The amount of cellular transfer via this anastomosis between abdominal cavities has not been determined, but there is evidence that it affects the outcome of parabiotic union (15).

Cross-Circulation: Capillary proliferation and vascular union between the two parabionts can be easily documented (9, 10, 16, 17). The rate of cross-circulation of blood can be measured by injecting isotope-labeled

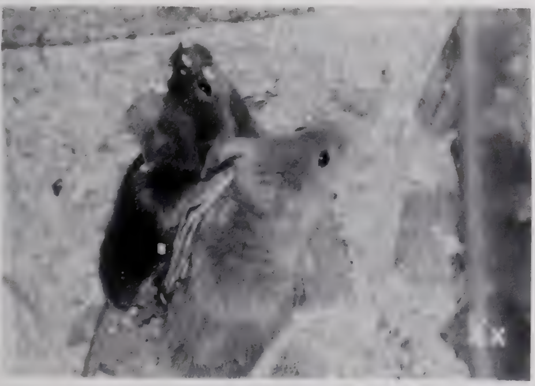

FIG. 2. DBA mouse (tan) parabiosed to (DBA/J × C57BL/6/J) F-1 hybrid at four weeks postsurgery.

erythrocytes into one partner and measuring the rate of disappearance of labeled cells from the injected animal and the rate of uptake of labeled cells in the noninjected partner. Blood samples are drawn in microhematocrit tubes at definite time intervals and radioactivity is measured in a scintillation counter. The rate of cross-circulation is calculated in terms of percent of total blood volume transferred per hour by the method of Bichel (16) as modified by McBride (17). By the fifth day, significant rates of exchange have developed syngeneic, semi-allogeneic and allogeneic parabiosed pairs, unless the animals have been presensitized (18, 19). By the seventh day, syngeneic pairs develop and maintain indefinitely a cross-circulation rate exceeding one hundred percent of the blood volume for one hour (17).

In semi-allogeneic and allogeneic pairs, the duration and extent of cross-circulation vary with the histocompatibility differences between the strains involved (10). Thus, after a period of time, the rate of cross-circulation may decline until there is no apparent crossover of cells; it is thought that the parabionts are no longer physiologically joined but are held together only by scar tissue (8).

However, the skin edges of the two parabionts do not break down, necrose or show signs of separation (18). Also, if blood samples are drawn 24 hours after cell injection and radioactivity measured, in some pairs a significant amount of labeled cells is found in the noninjected partner indicating that some cross-circulation may persist (14).

Polycythemia-Anemia (PA) Syndrome

Parabiotic intoxication is also manifested by a syndrome in which one mouse develops polycythemia and its partner develops anemia (Fig. 3) (7, 10, 20). The polycythemia may be manifested by a hematocrit as high as 80% and the anemia by a hematocrit as low as 10 or 15% (10, 14). Transfusion does not alleviate the anemia (1). In the semi-allogeneic model, the parental mouse usually, but not always, develops the polycythemia and the hybrid mouse the anemia (7, 10). In allogeneic models, one strain also consistently develops the polycythemia and the other the anemia (7, 10). For example, mice of the A/Jax strain become polycythemic when parabiosed to either CBA (4) or C57BL/6 (12) but when parabiosed to strain BALB/C they become anemic (7). Generally, strains that have only minor histocompatibility differences have PA syndromes of shorter duration and of lesser magnitude (10). Strains with major loci (H-2) differences have marked polycythemia-anemia shifts which may never abate.

The polycythemia-anemia syndrome evolves in two distinguishable phases (22). The early phase begins about day 5 and usually ends by day 10. The late phase begins during the second week and may be permanent, depending upon the strains involved (Fig. 3). There may also be a reversal of the direction of cell shifts in the late phases. Syngeneic parabionts may develop a transient early phase but never the late phase (10). A very late reversal or third phase has been observed in several long-living parabiont pairs in our laboratory (14).

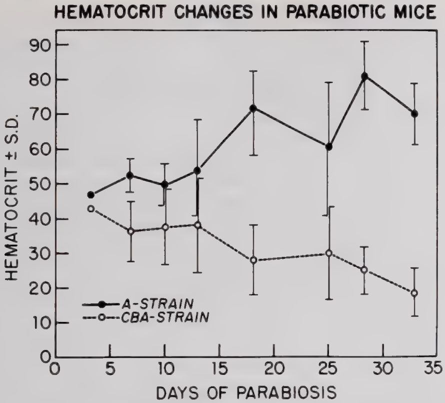

FIG. 3. Hematocrit changes occurring in parabiosis of CBA/J mice to A/J mice. The A mouse almost always becomes polycythemic and the CBA mouse anemic.

The pathogenesis of the PA syndrome is unknown but is assumed to be the consequence of an immunologic vascular reaction (10). If red cell radioisotope labeling studies are performed and samples taken after 24 hours, the polycythemic animal can be shown to have accumulated more of the tagged erythrocytes (14). For example, 24 hours after labeled CBA red cells are injected into a CBA mouse parabiosed to an A strain mouse, the A mouse accumulates more labeled cells then the CBA mouse (Table I) (14). If the A mouse is injected with labeled CBA erythrocytes or A erythrocytes, the equilibrium is initially directed toward the CBA mouse but then reverses and the A mouse again accumulates the cells (9, 14).

Erythropoiesis is not suppressed in the anemic animal's marrow; some animals show reticulocyte counts as high as 20% or more (10, 14). Reticulocytes are also present in the polycythemic partner, but it is unclear as yet whether these cells have cross-circulated from the anemic partner. However, marrow culture studies currently in progress suggest the presence of a serum factor that is capable of suppressing erythropoiesis in the anemic animal and that is not active in the polycythemic animal.

Thus, in the PA syndrome, a responsive marrow exists in an anemic animal which paradoxically loses cells to its polycythemic parabiotic partner. Immunologic factors have shifted an equilibrium that is physiologically balanced in syngeneic models (10).

Tolerance

The transplantation immunologist has been interested in parabiosis since it has been shown that parabiosis can induce immunologic tolerance specific for

TABLE I

Cross-Circulation Studies Showing More Than 50% 24-Hour Uptake of Cr51-Labeled Erythrocytes in Polycythemic A Mice Parabiosed to CBA Mice at 1, 2, and 4 weeks. The CBA Was Anemic and Was Injected with Labeled CBA Cells. Different Parabiotic Pairs Were Used for Each Determination

	1 Hour Cr51 Counts		24 Hour Cr51 Counts	
	CBA	A	CBA	A
		1 Week		
Pair 1	9588	6711	6334	8842
2	8624	4088	4531	7489
3	8715	7917	6178	8603
4	8387	6572	5623	8021
		2 Weeks		
5	6819	2341	2641	4312
6	11187	1238	3379	5733
7	9432	745	2308	3790
8	8716	824	2741	5214
		4 Weeks		
9	4124	2327	1429	3029
10	3187	1516	1217	2137
11	2403	2072	834	1715

the strains of the parabionts involved (10, 23). In a series of experiments (24–27) beginning with animals of the same strain but differing in sex and proceeding stepwise to parabiosed strains of greater immunologic disparity, they were able to show that skin allograft tolerance could be achieved and was transmissible by injection of lymphoid cells (28). Third-party skin is rejected in normal time periods. The duration of parabiotic union needed to induce tolerance varies with the strains parabiosed (27). With some models, tolerance is achieved only after a few weeks of union, while in others 10 weeks of union may be necessary (17). With some strains, tolerance is not achieved even after 20 weeks of union (17).

Tolerance induced by parabiosis has been shown to be transferable (28). If spleen cells from a parabiont in which tolerance to its partner has been induced are injected into syngeneic neonates, these neonates develop normally. If on maturity, the neonates are grafted with skin from animals syngeneic to the parabiotic partner, a permanent acceptance of skin grafts occurs. The duration of parabiotic union necessary to achieve transferable tolerance is longer than that needed to directly induce tolerance in the parabionts.

Martinez equates the development of transferable tolerance with the accumulation of cells from the partner in the spleen of the animals from which tolerance is transferred (28). Tolerance in the neonatal mice or the parabiont mice is a result of the amount of foreign antigen present. The time lag between the induction and transfer of tolerance is the time needed to accumulate a sufficient number of antigenic cells in the spleen, so that a substantial amount of foreign antigen is transferred. Lymphoid cell transfer has been analyzed in semi-allogeneic parent to F-1 parabiosis using a strain with a lymphocyte marker chromosone such as the CBA/T6T6 translocation. Examination of the F-1 hybrid spleen reveals a large number of parental cells (29), but only small numbers of F-1 hybrid reach the parental spleen during the same time.

A second possible mechanism of tolerance induction and transfer is the generation of "suppressor" lymphocytes as a consequence of the parental immunologic attack against the F-1 partner. The unresponsive state is caused by the presence of a humoral blocking factor produced by parental suppressor cells (1). With time, parental cells retaining the ability to produce this factor accumulate in the parental spleen. The unresponsiveness may thus be transferred to syngeneic mice by viable cells capable of producing this factor.

In mixed lymphocyte cultures of spleen cells obtained from tolerant parental strain animals bearing long-surviving F-1 skin grafts, reactivity still exists toward F-1 cells (32). This is in agreement with the concept that in mice, tolerance is dependent upon the presence of a blocking factor and not the elimination of the responsive clone of cells which is demonstrable by in vitro techniques.

Further studies of parabiosis-related tolerance will certainly shed light on the mechanisms of immunologic self-recognition, and will contribute to the development of newer modes of specific immunosuppression for organ transplantation.

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Profunda Femoris Revascularization: A New Technique

JEFFREY S. FREED, M.D., AND FABIO GIRON, M.D.

The importance of the profunda femoris artery (PFA) in maintaining circulation to the distal lower extremity in cases of superficial femoral artery (SFA) occlusion has been recognized in the past. The anatomy is such that there is excellent proximal anastomosis between the PFA system and the gluteal and deep circumflex vessels, and distally between the geniculate vessels and the tibial vessels (1). It is a common observation that an intact profunda system can provide an adequate vascular thoroughfare to the distal leg. The case presented demonstrates a simple surgical technique of profunda revascularization which can be used in patients as an alternative to other, more extensive, procedures.

Case Report

On November 9, 1974, a 62-year-old man was admitted to The Mount Sinai Hospital Vascular Surgical Service with a chief complaint of progressive intermittent claudication of the left calf, and recent onset of rest pain and numbness of the left foot. He had no history of diabetes mellitus, hypertension, cardiac or cerebrovascular disease, or smoking. The patient had previously undergone left lumbar sympathectomy (1960), right lumbar sympathectomy (1961), right above-knee amputation (1967), and left aorto-femoral bypass and left anterior compartment fasciotomy (1967). A left groin infection developed postoperatively requiring incision and drainage, leaving the dacron prosthesis exposed. Careful treatment resulted in complete healing after two months. The patient had been successfully rehabilitated and was able to walk with a prosthesis until two months prior to admission when he developed the above symptoms. Physical examination revealed an ischemic left foot with dependent rubor, elevation pallor, poor capillary filling, and mottling of toes. There were no pulses below the left femoral. Right femoral pulse was absent. Both radial and carotid pulses were strong. There was a large abdominal incisional hernia and extensive scarring of the left groin incision area.

A translumbar aortogram was performed which revealed patency of the aorto-femoral bypass, occlusion of the SFA 10 cm distal to its origin, 80% occlusion of the orifice of the PFA, no popliteal reconstitution with poor runoff (Fig. 1).

In view of the threat of gangrene in the patient's remaining leg, it was decided to attempt to revascularize the PFA as a limb salvage procedure.

At operation the left groin incision was avoided because of the extensive scarring resulting from the previous infection and the presence of the dacron prosthesis. The PFA was exposed 5 cm below its origin and found to be a soft, pliable, pulseless vessel. The superficial femoral artery was then exposed at the same level as the PFA and was found to have a strong pulse. A bypass was then constructed between the SFA and the PFA at this level, using a short segment of saphenous vein. A strong pulse was restored in the PFA after the anastomoses were completed.

Postoperatively, the patient had immediate relief of his rest pain and the foot appeared warm with good capillary filling. A translumbar aortogram was performed on the third postoperative day in order better to visualize the distal circulation. The study revealed patency of the graft, and runoff consisting of a fair posterior tibial artery, suitable for further reconstructive surgery if

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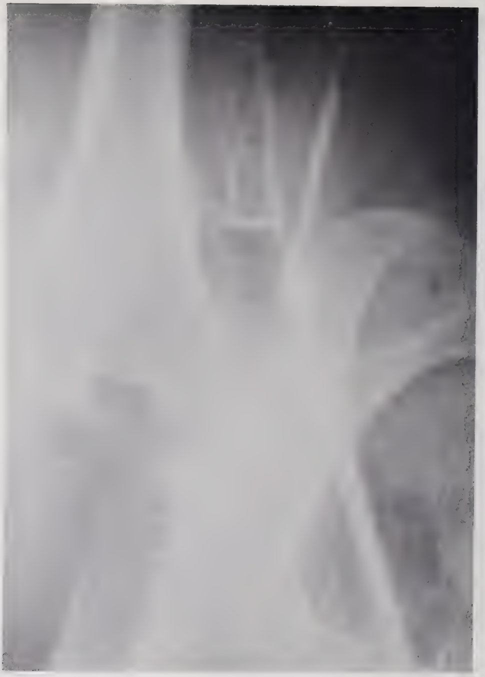

FIG. 1. Admission angiogram demonstrating stenosis of origin of PFA.

necessary. Marked increase in profunda circulation was noted (Fig. 2). However, when the patient resumed walking with his prosthesis, he was able to go more than 100 yards without claudication.

Discussion

Operable lesions of the profunda femoris are present in a significant percentage of patients with superficial femoral disease (2). In multiple recent reports, techniques of profundaplasty, distal profunda revascularization, and profunda orifice endarterectomy have been described (2–5). Revascularization of the PFA alone has given good results in salvage of ischemic limbs where femoro-popliteal bypass was not considered feasible. Furthermore, in selected cases, profunda revascularization may be preferable to the standard femoro-popliteal bypass (3).

The profunda bypass described is suitable for the anatomic situation where the proximal SFA is patent. It is a useful, simple technique of profunda revascularization that may be employed for limb salvage with minimal risk to the patient.

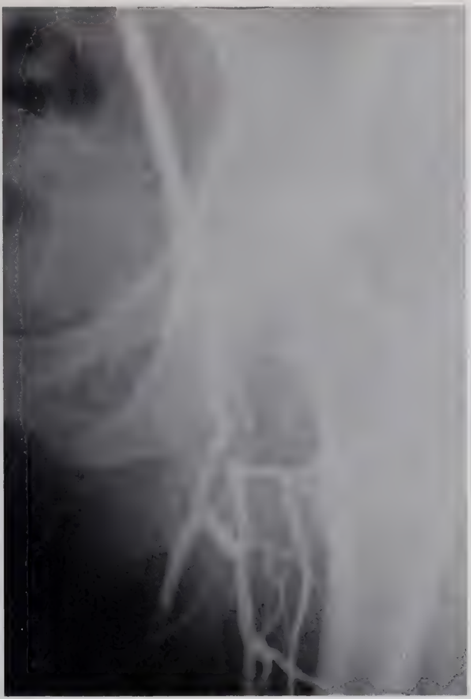

FIG. 2. Postoperative angiogram demonstrating increased distal PFA flow.

Summary

A patient with an extremity threatened by ischemia is presented. The importance of the profunda femoris artery is discussed in regard to distal limb circulation. A technique for profunda revascularization is described using saphenous vein to direct the proximal SFA flow to the PFA.

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A Phytobezoar in an Adult

MARK KLEIN, M.D., SE-MIN BAEK, M.D., AND UNSUP KIM, M.D.

Bezoar of the stomach in adults is rare, and is usually seen among patients in mental institutions and in postgastrectomy patients.

A case of phytobezoar in a vegetarian, associated with benign gastric ulcer is reported.

Case Report

A 73-year-old retired Israeli man was admitted to The Mount Sinai Hospital with a one-month history of epigastric pain radiating to the back and substernal area. The pain was relieved by intake of food. Dietary history was significant in that the patient was a vegetarian for 10 years prior to admission. He consumed approximately 25 oranges, 6–10 grapefruits, 10 persimmons, and a large quantity of uncooked fresh vegetables each day. Medical history was unremarkable.

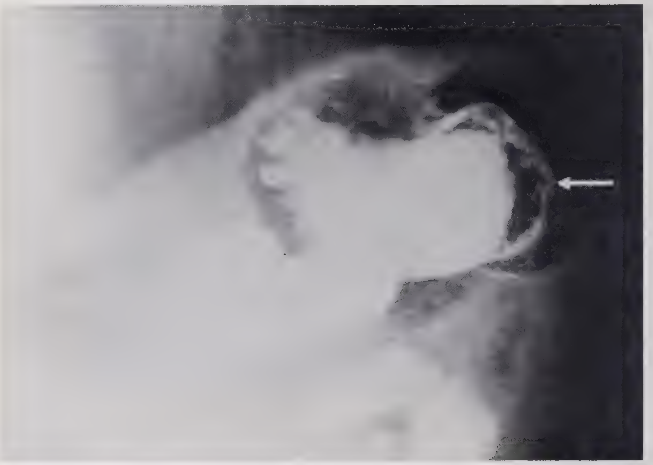

FIG. 1. Upper GI Series showing bezoar which is partially coated by barium meal (arrow).

Physical examination showed a healthy elderly male with normal vital signs, and entirely normal physical findings.

Upper gastrointestinal series showed a large filling defect in the stomach (Fig. 1), and an ulcer in the prepyloric region along the lesser curvature. Gastroscopic examination showed a large bezoar in the cardia of the stomach and a 2-cm benign ulcer in the prepyloric region.

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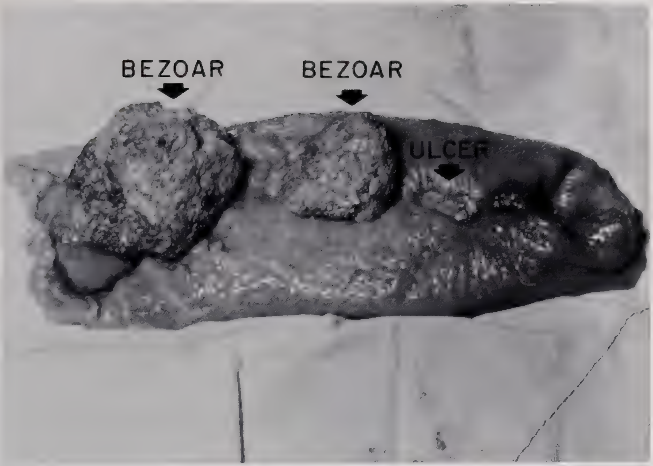

FIG. 2. Specimen showing two bezoars and ulcer.

On laparotomy, two large bezoars and a deeply penetrating prepyloric ulcer were found in the stomach (Fig. 2). A bilateral truncal vagotomy, antrectomy, and Billroth I gastroduodenostomy was performed. The postoperative course was uneventful.

Comment

The clinical manifestations of bezoar are nonspecific and are difficult to distinguish from those of other gastrointestinal ailments. Frequent presenting symptoms include anorexia, nausea, and vomiting, associated with epigastric pain. When there is ulcer disease associated with the bezoar, characteristic symptoms are apparent.

Reviewing 311 cases of bezoars and concretions, DeBakey and Oschsner in 1938 (1) reported that 40% were phytobezoars. Citrus fruit and other foods high in fiber content have composed most of the bezoars analyzed.

Of those phytobezoars found in patients without prior surgery, the persimmon has been implicated as the cause in approximately 85% of cases (1, 2). The persimmon is a plum-like fruit and contains phlobotannin, which is precipitated by gastric juice. This sticky precipitate serves as a glue to bind the fruits, skin, and seeds together.

Because phytobezoars are often found in elderly patients, quite frequently the radiographic findings may be diagnosed as cancer of the stomach, especially when ulcer is associated. Endoscopic examination will be diagnostic. The most common complication associated with gastric bezoars are gastric ulcer and intestinal obstruction. The reported incidence of intestinal obstruction was 26.5%, and ulceration 24.4% (1). Surgical removal remains the treatment of choice, although successful medical therapy for bezoars occurring in postgastrectomy patients has been reported (3).

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ATTENDINGS' SECTION

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Lymphosarcoma of the Large Bowel

RICHARD H. MARSHAK, M.D., DANIEL MAKLANSKY, M.D., ARTHUR E. LINDNER, M.D., AND BERNARD S. WOLF, M.D.

It is unusual for the large bowel to be involved with lymphosarcoma, either as a localized tumor or as part of systemic lymphoma. As a primary lesion, lymphosarcoma accounts for only 0.5% of all malignant tumors of the colon and 0.1% of all malignancies of the rectum (1). In this paper we are concerned with primary lesions involving the colon. Extrinsic pressure on the colon from lymphosarcoma of adjacent organs and tissues may also occur.

Within the large bowel, the disease tends to develop in the cecum and in the rectum. Both small bowel and colon may be simultaneously involved (2), and on rare occasions stomach, small bowel and colon are diseased. On gross pathologic examination the tumor appears as an ulcerated or polypoid mass, resembling a carcinoma, or as a diffuse process extending over a large segment or even the entire colon. The diffuse tumors demonstrate thickening of all bowel layers, with nodularity of the surface, which may be ulcerated. Regional lymph nodes are involved in approximately half the cases at laparotomy and the lesion may extend beyond the colon wall as a bulky extracolonic mass.

The diffuse form of lymphosarcoma of the colon is characterized pathologically by extensive thickening of the submucosa as a result of infiltration with innumerable, closely packed, round cells. It is assumed that this process starts in the submucosal lymphoid follicles, which in places extend into the mucosa. It is not known whether it begins as a multicentric process or arises from a single area, later spreading by direct extension or through lymphatic channels. When patients with the diffuse type of lymphosarcoma of the colon are first seen, a large part of the colon may be involved in a uniform and continuous fashion. Submucosal infiltration extends for some distance from the area of obvious gross involvement and there may be additional lesions apart from that region. Marked involvement is most common in the ileocecal or the rectosigmoid areas, where the tumors sometimes become confluent and form a large conglomerate mass. This may cause intussusception and incomplete intestinal obstruction. In the ileocecal region there is ordinarily extension of the process into the appendix and into the ileum for a variable distance. When the rectum is the site of the tumor, inguinal nodes may be enlarged and palpable. Extensive serosal or retroperitoneal involvement is not characteristic of this variety of lymphosarcoma.

On palpation, the bowel wall is thickened and rubbery in consistency. The cut surface of the broad submucosa is homogeneously grayish-white, with few blood vessels. The most striking changes are observed when the mucosal

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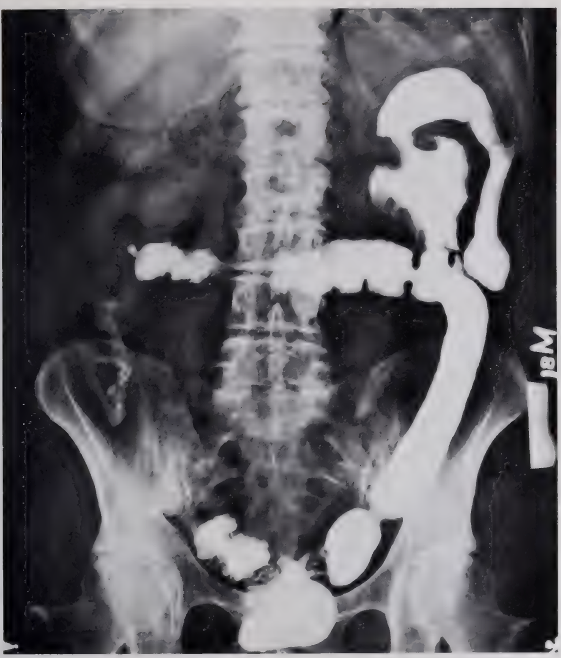

FIG. 1. *Lymphosarcoma of the colon.* A discrete mass is present in the region of the splenic flexure. This measures approximately 3½ inches in length with moderate narrowing at each end. The central portion of the mass is more dilated than the actual lumen of the bowel. The contours are irregular, scalloped, secondary to pressure from tumor. It is difficult to state whether the dilatation is due to a large ulceration or to aneurysmal dilatation of the bowel wall.

aspect of the colon is inspected. The mucosa is thrown up into a huge number of convoluted folds which resemble the surface of the brain and individually may reach a thickness of one or two centimeters. Superimposed on the thick convolutions are innumerable broad-based intraluminal protrusions. Although these may be termed pseudopolypoid, elongated, thin pedicles are rare. Deep ulceration and perforation are uncommon, in comparison with disease in the small bowel although areas of superficial mucosal necrosis may be present.

In most reported series (3, 4) the incidence is greater in males than in females, in a ratio of 2 to 1. The majority of patients are over 50 years old at the time of diagnosis but all age groups are represented. Most patients with colon

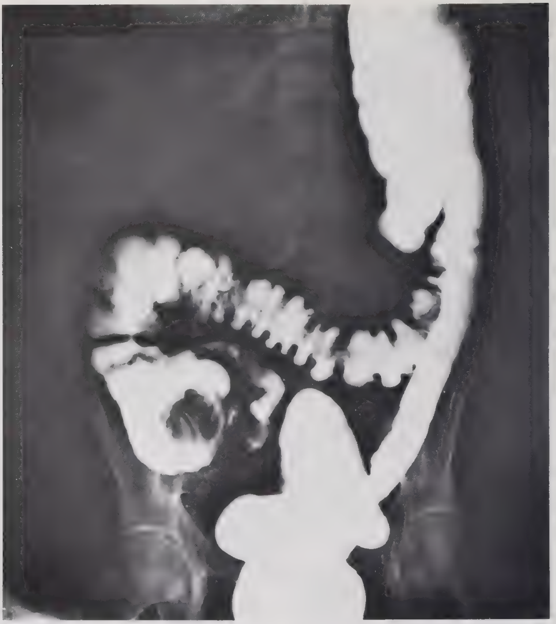

FIG. 2. *Lymphosarcoma of the colon.* There is a slightly lobulated polypoid tumor mass measuring 3 cm in diameter in the cecum. A single diagnosis is impossible in this case. A

lymphosarcoma have abdominal pain, usually cramping in quality and localized to the area of the tumor. Other prominent clinical findings include weight loss, change in bowel habit, weakness, nausea and vomiting, anorexia, and fever. Gross rectal bleeding and severe anemia are uncommon. Intussusceptions occur (5) and cecal lesions may be palpated on abdominal examination. Discrete intraabdominal masses are usually not appreciated until late in the disease process. When the rectum is involved, presenting complaints include bleeding, diarrhea, constipation and tenesmus. The lesion is frequently palpable on digital examination of the rectum and occasionally a tumor mass protrudes from the anus.

Controlled studies of treatment methods in colon lymphosarcoma are not yet available, but initial efforts are directed at cure by surgical resection, usually followed by local radiation therapy. In one series of patients so treated (3), a five-year survival rate of 55 percent was reported, but the relative effectiveness of surgery and radiation is not clear. In tumors that are unresect-

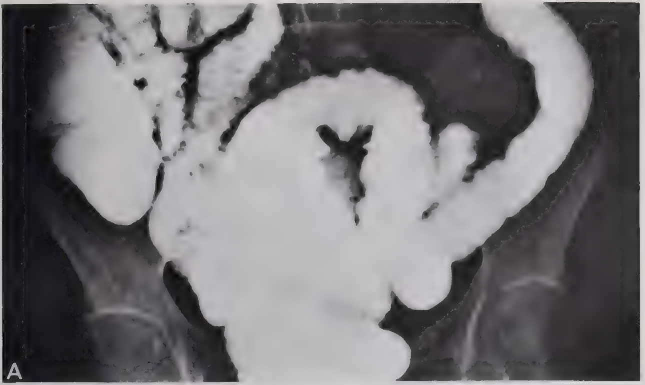

FIG. 3A. *Lymphosarcoma of the colon.* Multiple, small, scalloped defects are seen along the contour of the bowel. There is no evidence of spasm, irritability, or an inflammatory process. The diagnosis was made on sigmoidoscopy where multiple, small, mound-like structures without ulceration were identified.

able, radiation therapy is of definite benefit, and systemic disease is treated by combined programs of radiation and chemotherapy.

Roentgen Features

Lymphosarcoma of the colon occurs in two principal forms: one is a discrete or localized type that presents as a single mass; and the other is an extensive, diffuse, infiltrating tumor that extends over long segments of the bowel (6).

The localized variety of lymphosarcoma may present as an area of narrowing with little or no obstruction, with the roentgenographic changes of carcinoma of the colon, and the pathologic diagnosis often comes as a surprise. In another form of localized lymphosarcoma, aneurysmal dilatation of a short segment of colon occurs (Fig. 1). Such aneurysmal dilatation has two causes. First, the absence of a desmoplastic process allows a malignant ulcer to become huge; and secondly, lymphosarcoma destroys the muscular layer and its neurogenic plexuses, producing a localized aneurysm of the bowel at the involved site. In a third type of localized lymphosarcoma, there is a single polypoid mass indistinguishable from polypoid carcinoma. This type is most frequently seen in the cecal area (Fig. 2). In considering all these localized forms, it should be noted that at times lymphosarcoma extends through the bowel, producing a mass with both an intraluminal and an extramural component.

In the diffuse type of lymphosarcoma, the entire colon or long segments are involved. (Figs. 3–7) Frequently the process is associated with concomitant disease of the terminal ileum. In its earliest form small discrete nodules are

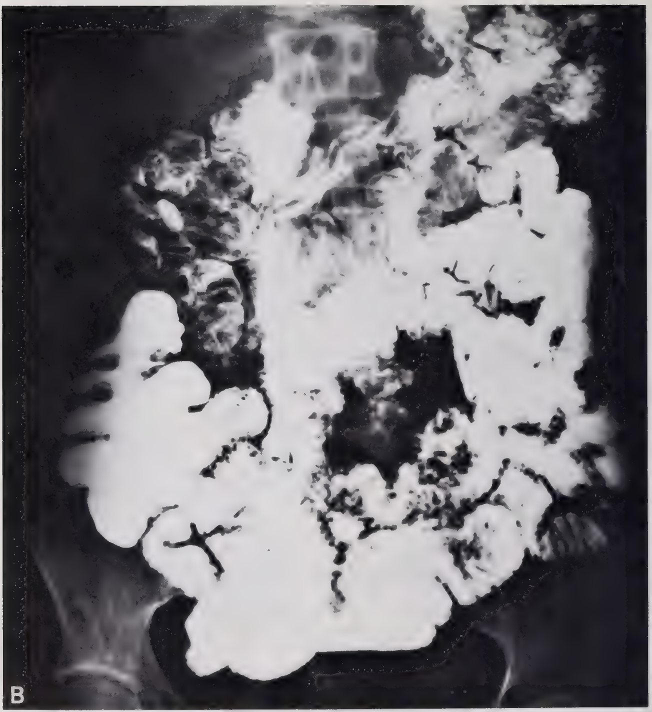

FIG. 3B. *Lymphosarcoma of the small bowel.* Same patient as Figure 3A. Multiple nodules of varying size are identified in the proximal ileum. The diagnosis was confirmed by biopsy. The patient improved after radiotherapy and is alive and well five years later.

seen along the contour of the bowel, appearing as scalloped, irregular filling defects. These may be found throughout the colon or confined to a short segment. When the bowel is fully distended with barium the nodules may be partially effaced, but usually some evidence of these small contour defects can be detected.

In more advanced disease, barium enema examination shows a transformation of the mucosal pattern into an extremely irregular, nodular appearance with many coarse folds and finger-like projections into the bowel lumen. These findings are most apparent on evacuation films. The tumor masses and the lack of desmoplastic reaction cause the colon to be more distensible than

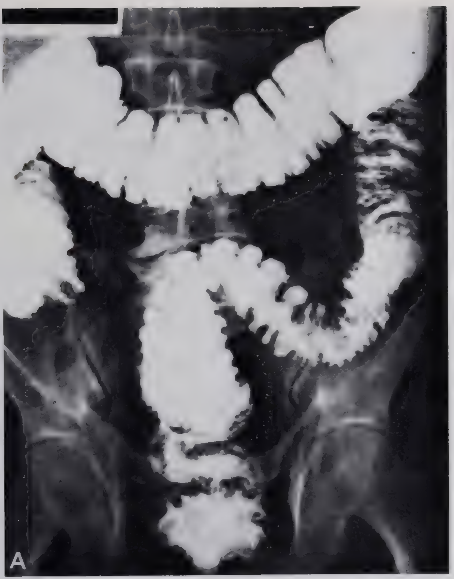

FIG. 4A. *Lymphosarcoma of the colon.* Multiple large, irregular folds are seen in the rectum and sigmoid associated with intraluminal defects. There is no evidence of spasm, irritability, or shortening of the colon. The bowel appears to be more distensible than is usually noted, probably secondary to the tumor masses. The entire colon is involved.

would be anticipated. For similar reasons the bowel fails to collapse after emptying. Despite the presence of so many tumors and the large irregular folds, there is little irritability or spasm of the bowel during the introduction of barium. The haustral pattern tends to be maintained at this stage, the bowel is not shortened, and there are no intraluminal secretions or mucosal ulcerations. When the bowel is completely filled, the intraluminal projections may be obscured, although numerous closely spaced polypoid indentations persist along the contours. Pedicles can rarely be identified in the polypoid tumors of lymphosarcoma.

As the process continues and submucosal tumor becomes extensive, the haustral pattern disappears and is replaced by large irregular folds resembling giant rugae in the stomach. Such thick folds are not easily effaced but they can be obscured if the colon is distended. In these instances the evacuation film is once more extremely important.

Intussusception, if present, is ordinarily easily reduced, although a mass may persist in the region of the ileocecal valve and prevent filling of the terminal ileum (Fig. 8).

In the final stages of lymphosarcoma the folds may become so large and bulky that complete filling of the colon is difficult and areas of bowel obstruction secondary to tumor may be encountered (Fig. 6).

Lymphosarcoma involving small bowel, mesentery or regional lymph nodes may produce large, bulky masses causing extrinsic compression on the colon (Fig. 9).

Differential Diagnosis

From the roentgen point of view, diffuse lymphosarcoma of the colon must be differentiated from familial polyposis, ulcerative colitis with pseudopoly-

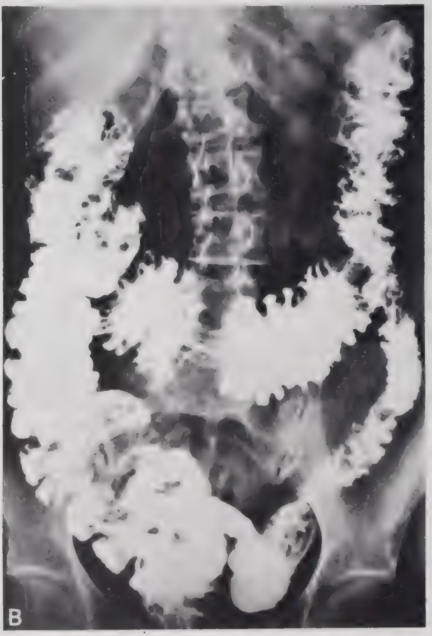

FIG. 4B. *Lymphosarcoma of the colon.* Evacuation film of Figure 4A. The bowel still retains a considerable amount of barium. Multiple finger-like projections are seen extending from the contour of the bowel associated with small defects. Numerous small polypoid lesions are also present.

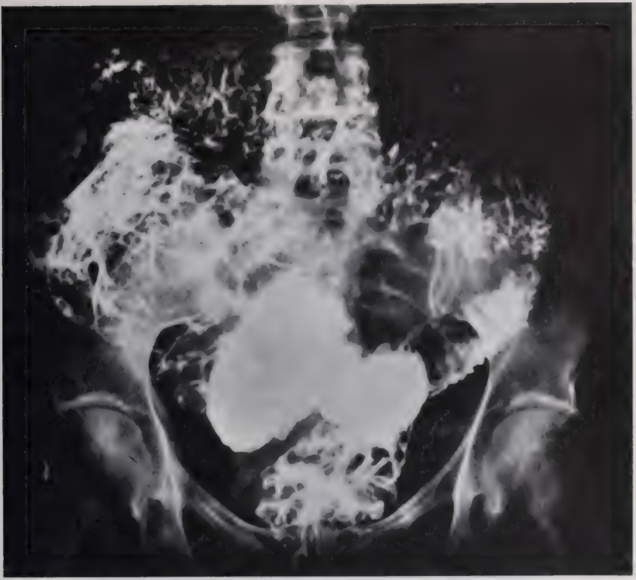

FIG. 5. *Diffuse lymphosarcoma of the colon, evacuation film.* The size of the colon remains large despite the evacuation of most of the barium. The mucosal pattern is reticulated. Multiple nodules of varying size are identified.

poid changes, granulomatous colitis, nodular lymphoid hyperplasia, and schistosomiasis.

In contrast to familial polyposis, the polypoid changes in lymphosarcoma are associated with thickened folds and frequently with large tumor masses, especially in the region of the cecum. Simultaneous extensive involvement of the ileum and the colon is uncommon in familial polyposis. In early cases differentiation may depend on sigmoidoscopy and biopsy.

Unlike ulcerative colitis, diffuse lymphosarcoma shows no ulcerations, the haustral pattern is preserved; and shortening, irritability, and deformity characteristic of the former disease are absent.

Granulomatous ileocolitis may also present as a diffuse nodular thickening of the mucosa and submucosa. In ileocolitis, there is eventually narrowing and deformity of the bowel with mucosal ulceration, irregular shortening, and eccentric pseudodiverticular formation.

Benign lymphomas of the colon are rare and present as single or several

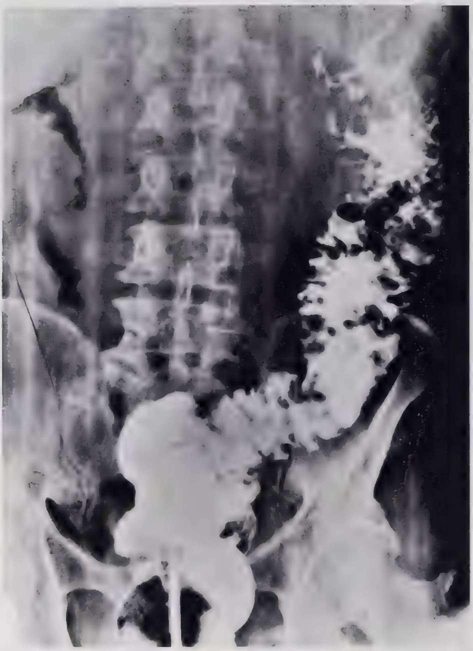

FIG. 6. *Lymphosarcoma of the colon and small bowel.* There is a complete obstruction in the region of the mid descending colon due to the presence of a large number of huge tumor masses. The contour of the bowel is irregular. Also noted is the narrow and irregular contour of air filled loops of bowel on the right side of the abdomen due to involvement of the small intestine with lymphosarcoma.

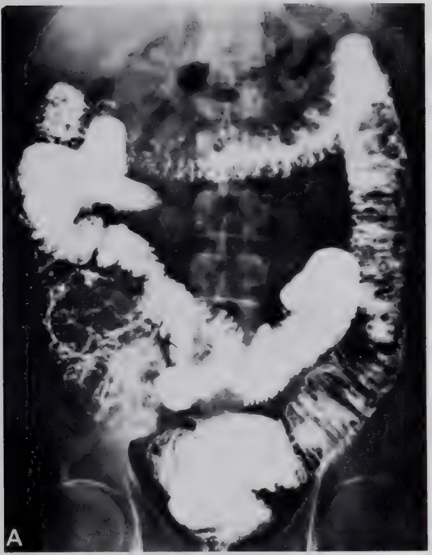

FIG. 7A. *Lymphosarcoma of the colon.* A remarkable case of diffuse lymphosarcoma mimicking familial polyposis. There are numerous polyps distributed throughout the colon. A large polypoid tumor mass is identified in the cecal region. This type of tumor mass is more commonly seen in lymphosarcoma than in familial polyposis.

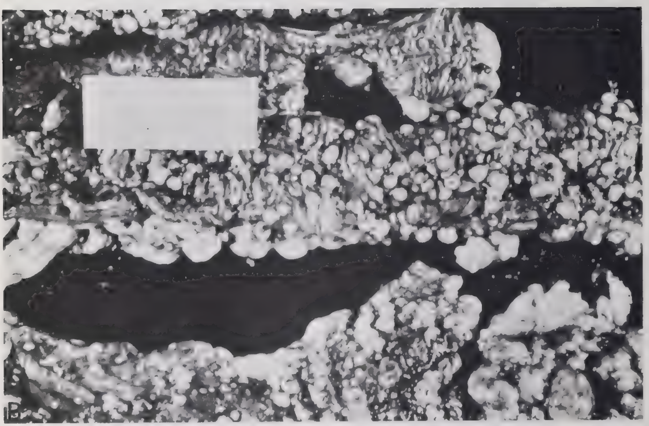

FIG. 7B. *Lymphosarcoma of the colon*. Same case as Figure 7A. The specimen bears a remarkable rsemblance to familial polyposis. On a superficial examination, differential diagnosis is impossible. The presence of the large mass in the cecum suggests lymphosarcoma. The polyps in this case are also of interest because they all proved to be pedunculated.

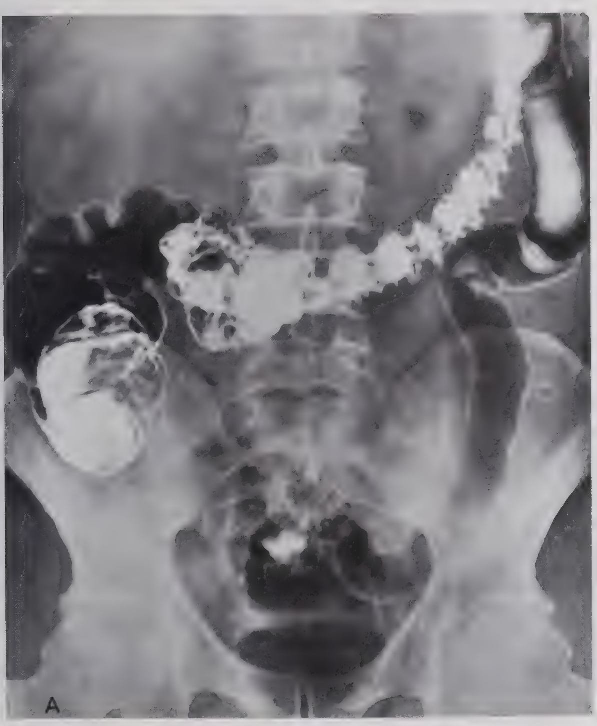

FIG. 8A. *Lymphosarcoma of the colon.* There is a polypoid mass in the cecum and ascending colon due to intussusception of a polypoid lymphosarcoma of the terminal ileum into the right side of the colon.

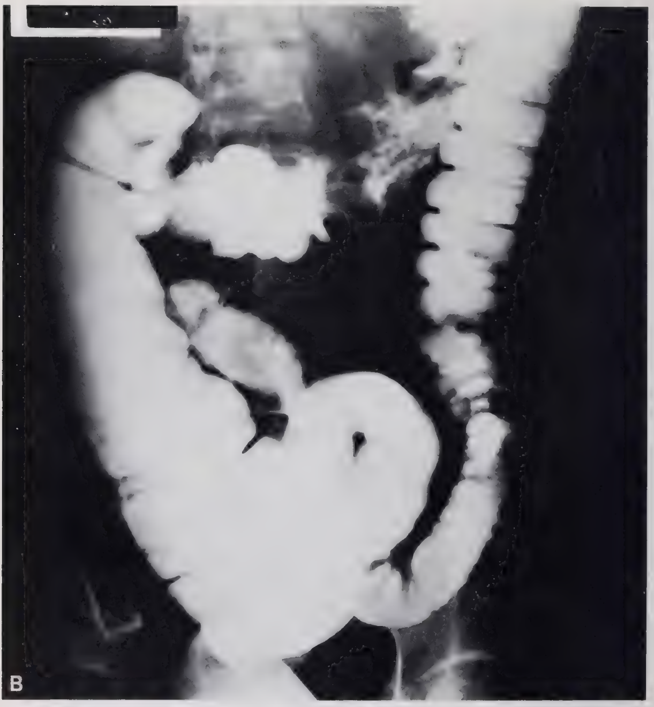

FIG. 8B. *Reticulum cell sarcoma of the colon.* The distal transverse colon is spastic, irritable, and markedly irregular. The folds are distorted, thickened, and have a wild configuration. No discrete tumor masses are identified. A single diagnosis was difficult in this case. The following possibilities were considered: granulomatous colitis, pancreatitis, metastatic carcinoma, and lymphosarcoma. The final diagnosis was recticulum cell sarcoma. This is a young man in his late 20's who had an appendectomy six weeks prior to his present admission. He was reinvestigated because of persistent fever.

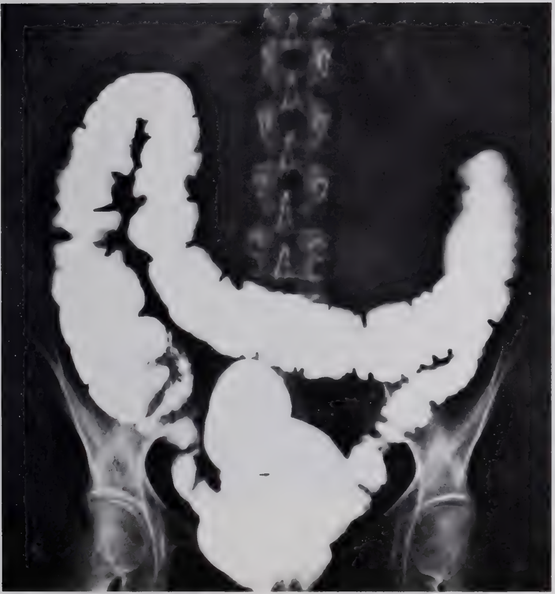

FIG. 8C. *Lymphosarcoma of the colon.* Multiple small nodular contour defects are seen on the right side of the colon. The following entities were considered in differential diagnosis: neurofibromatosis, metastatic carcinoma, and lymphosarcoma.

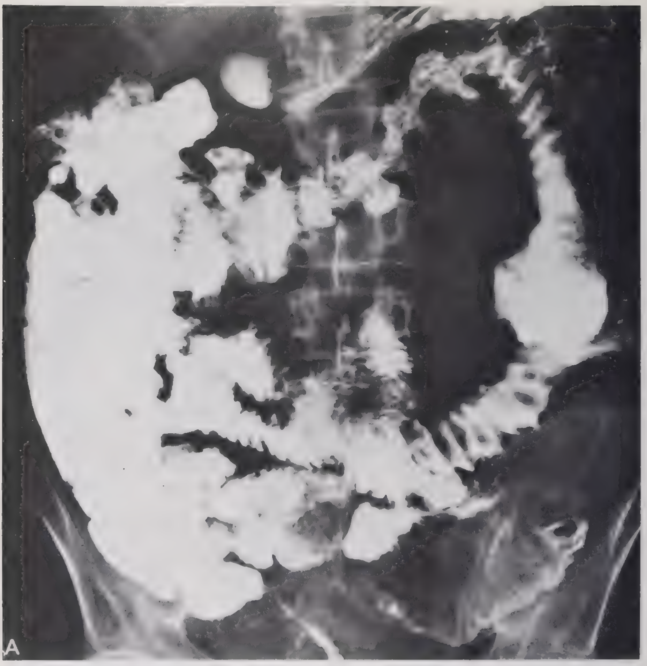

FIG. 9A. *Lymphosarcoma of the small bowel.* A large ulcer is identified with irregular, scalloped borders. Proximal and distal to the ulcer are thickened perpendicular rigid folds. A large irregular ulcer crater in the small bowel is almost pathognomonic of a sarcoma but it can also be seen with metastatic carcinoma.

polypoid tumors of the rectum and sigmoid. A specific roentgenogrphic diagnosis is impossible (7).

Nodular lymphoid hyperplasia of the colon usually occurs in infants and children (8). The defects are tiny and may be so numerous that familial polyposis is simulated. An inflammatory reaction is not seen. There is no ulceration or thickening of the folds. An early case of lymphosarcoma may be difficult to distinguish from nodular lymphoid hyperplasia. Serum immunoglobulin defects have been reported in the latter disease.

Schistosomiasis (9) in endemic areas, and especially in Egypt, may produce large inflammatory polyps throughout the colon. Although the filling defects may simulate lymphosarcoma, submucosal nodules and contour defects are absent. An inflammatory process involving the entire colon is frequently present, indicated by absence of haustral markings and by some degree of

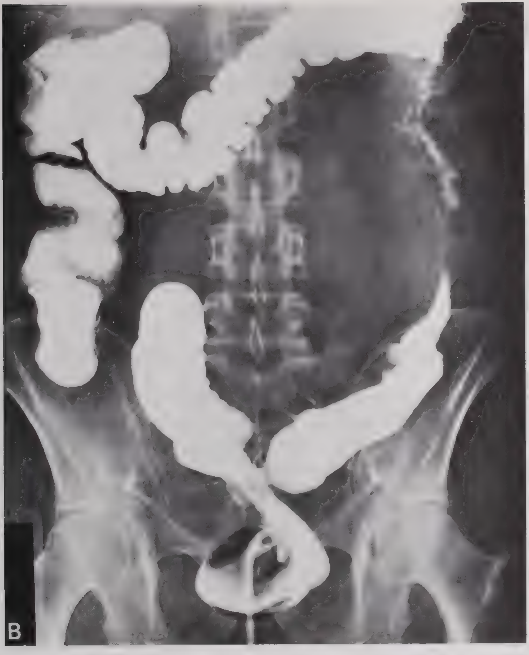

FIG. 9B. *Lymphosarcoma of the small bowel.* Same case as Figure 9A. The large mass surrounding the ulcer has produced extrinsic pressure on the left side of the colon.

narrowing and shortening of the bowel. Tumor masses are not seen. Increased secretions are usually present.

Hodgkin's disease involving the colon is so rare that we have seen only three cases. One was associated with ulcerative colitis. The other two demonstrated marked narrowing, simulating metastatic carcinoma rather than lymphosarcoma. We have not seen a patient with primary Hodgkin's disease of the colon.

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Trasylol Revisited: The Value of Proteolytic Inhibitors in the Therapy of Pancreatitis*

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Introduction

Despite progressive improvement in management of patients with acute pancreatitis, particularly with respect to fluid and electrolyte control and recently described methods of removing active proteolytic enzymes from the peritoneal cavity and/or thoracic duct, the mortality in the hemorrhagic and necrotizing forms of pancreatitis still remains prohibitive. Although these fulminating forms of pancreatitis are relatively rare, when they occur all methods of treatment, including resection of the entire gland, may prove to be of little value.

With the objective of reducing the mortality of fulminating pancreatitis, pancreatic enzyme inhibitors of one form or another have been studied both experimentally and clinically during the past three decades. In 1926, Frey (1) demonstrated that the exocrine pancreas elaborated a hypotensive proteolytic substance. Twenty-four years later, in 1950, this proteolytic enzyme, kallikrein, was detected by Frey and coworkers (2) in blood and parotid gland in association with an inhibitor (3). In 1952, Werle (4) in Germany isolated an inhibitor of pancreatic kallikrein. This kallikrein-trypsin inhibitor, trasylol, is itself a polypeptide of molecular weight 11,500. Kallikrein is believed to be responsible for the production of bradykinin and other kinins in acute pancreatitis.

Bradykinin and other polypeptides have been incriminated in the pathogenesis of shock and myocardial depression in acute pancreatitis. Bradykinin and related polypeptides are derived from bradykininogen, a circulating precursor α-2-globulin produced in the liver. These substances are believed to contribute both to the local inflammatory pattern and the generalized systemic picture in fulminating disease. The physiologic effects of these "pallidins" include pain, hypotension, vasodilatation, and increased capillary permeability, the primary manifestations of pancreatitis (5). In addition, Webster and Clark (6) have implicated this system in the evolution of shock.

Experimental Studies with Trasylol. Early studies with trasylol carried out on experimental animals (7) showed this to be an effective proteolytic inhibitor if administered prophylactically or during the very early stages of

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disease. If used late during the course of pancreatitis, this agent was ineffective and failed to arrest the natural progress of the disease (8). Studies on cats showed trasylol to be ineffective, but the prophylactic administration was said to be effective (9). Similarly, despite the negative results of some authors (3, 10–12), utilizing dogs, early administration of the proteolytic inhibitors was found to be effective in ameliorating the effects of, and reducing the mortality of, experimentally induced acute pancreatitis (1, 10, 13–17).

Studies on Human Subjects with Trasylol. The therapeutic appeal of trasylol in human subjects derives from the fact that it is relatively harmless, and that intravenous injection may be given with no significant side effects.

Early studies on human subjects described immediate relief of pain and excellent clinical responses in individual patients. They were, however, carried out utilizing "homeopathic" doses of ±12,500 units which have since been proven to be ineffective in vivo, and inadequate in amount in vitro, to effectively neutralize the expected amount of trypsin liberated in acute pancreatitis. Goldberg and Roy (9) have demonstrated in their laboratory that the anti-proteolytic or anti-esterase activity of 1,000 units of trasylol inhibit approximately 1 mg of crystalline bovine trypsin. Yet the daily trypsin output computed from fecal and ileal content has a mean value equivalent to 125–228 mg of bovine crystalline trypsin. The minimum amount of trasylol administered in 24 hours should be able to neutralize an amount equal to the expected daily trypsin output—that is, 125,000–228,000 units. This was suggested by these authors as early as 1965, yet dosages as great as this have rarely been used. Early clinical studies (4, 14, 18–21) were either carried out on limited or small groups of patients, and were essentially uncontrolled. Larger clinical trials carried out during the following decade (1, 2, 22, 23), included varying types of pancreatitis, and, although including double blind studies, were poorly controlled, and failed to demonstrate adequately a statistically valid amelioration of acute pancreatitis.

Encouraged by the work of Moshall et al (9, 19) suggesting a higher dosage of trasylol in an amount sufficient to neutralize the massive quantities of proteolytic hormones released during the cellular destruction and necrosis of acute pancreatitis, Trapnell et al (24, 25) instituted a controlled clinical trial of trasylol. Their initial study in 1967 (24) utilizing a small daily dosage of 20,000 units had not shown a significant difference, *but* by contrast their second study published in 1974 (25) utilizing massive doses of the drug, 1,000,000 units the initial day followed by 800,000 units for four additional days, shows a statistically significant difference in mortality between the treated and untreated group of patients. They studied their patients at several stages during the course of the trial. When 100 patients had been treated the statistically significant difference at the 5 percent level became apparent. In the trasylol-treated group, 4 of 53 patients died, a 7.5 percent mortality. In the control (placebo) group, 13 of 52 patients (25 percent) died. Furthermore, there were more patients with mild disease in the treated group.

Let us look at Trapnell's data in Tables I and II, and Figure 1.

I have the following criticisms of this study:

1. The patients were restricted to biliary tract pancreatitis and idiopathic pancreatitis. Why was alcoholic pancreatitis, which is more severe and important, excluded? What was the distribution of each etiology in each group? Acute biliary tract disease is associated in at least 25 percent of cases with transient pancreatic edema, a clinically benign complication. Were such patients included?

2. Even by the subjective criteria used to grade the patients' severity of disease, the trasylol group is favored by milder case distribution.

3. I cannot understand or accept a mortality of 25 percent in a random series of pancreatitis. Not in this day and age. I have just completed a survey

TABLE I

Acute Pancreatitis (Severity of onset in relation to eventual clinical course)

Onset		Course			
Grade	Total	Mild	Moderate	Severe	Died
I	12	6	1	3	2
II	73	38	14	12	9
III	31	11	9	4	7
IV	12	4	2	3	3

TABLE II

Acute Pancreatitis (Severity of onset)

Grade	Control		Trasylol	
	No. of pts.	Died	No. of pts.	Died
I	3	1	2	
II	30	6	25	2
III	17	5	25	2
IV	2	1	1	

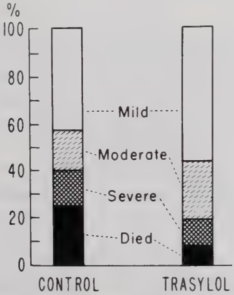

FIG. 1. Percentage composition of severity of pancreatitis in fatal cases of acute pancreatitis in patient treated or not treated with trasylol.

TABLE III

Summary of results of experimental studies using trasylol on various types of induced pancreatitis

Mechanism	Dosage q 4 hrs × 4 days	Treated/Control	Evaluation*
1. Ligation duct	10,000	12/12	No difference
Stimulation secretion	20,000	6/6	No difference
2. Typhoid injection	20,000	12/12	No difference
Duct ligation	40,000	6/6	No difference
3. Bile + trypsin	40,000	13/11	No difference
Duct ligation	50,000	6/6	No difference
	50,000†	6/6	No difference
4. Pfeffer	10,000	12/12	No difference
mechanism	50,000	5/5	No difference
	50,000†	5/5	No difference

* Based on length of survival, mortality %, gross and microscopic pancreatic pathology
† Prophylactic 24-hour treatment

TABLE IV

Summary of clinical results using trasylol prophylactically and therapeutically

Series	Treated/ Untreated	Mortality		Average Amylase Days*	Average Symptom Days†	Significance
		No.	%			
1. Acute alcoholic pancreatitis	62/59	1/1	1.6/1.7	2.3/2.5	4.3/4.1	None
2. Cholecystectomy	73/75	1/0	1.4/0	0.2/0.2	2.1/2.4	None
Gastrectomy	13/13	0/0	0/0	0.3/0.4	3.1/3.3	None?
3. Pancreatic resection						
Pancreatitis	8/7	0/0	0/0	1.3/1.4	4.0/3.0	None?
Pancreatic CA	3/5	0/1	0/20	1.8/1.6	4.0/4.0	None?

* Days with at least 1 elevated blood amylase
† Days with signs of peritonitis or ileus, fever, leucocytosis

of all patients with pancreatitis admitted to the following hospitals during my service at the various institutions: Harlem, Greenpoint, Elmhurst Hospitals, all municipal institutions, and Mount Sinai, Beth Israel, Joint Disease, and Bronx Veterans Administration Hospital. Among 10,263 cases treated for acute pancreatitis, there were 513 deaths or a mortality of 5 percent.

My studies with trasylol embrace many years of effort and include both experimental and clinical investigations. In the late 1950's, I was given a grant to study the effect of trasylol on canine pancreatitis. My results are summarized in Table III. These findings did not support usefulness of trasylol in experimental acute pancreatitis.

At the end of the 1960's I was given another large grant to study the value of trasylol in human pancreatitis. I had surveyed its effectiveness at a dosage level of 10,000 q.6.hr, and 20,000 q.6.hr., in a series of 27 and 32 cases of pancreatitis. My results did not encourage me to a controlled study. This time a protocol was set up to study three groups of patients:

1. Attacks of acute pancreatitis in the alcoholic—therapeutic.
2. Patients undergoing gallbladder surgery and/or gastrectomy—prophylactic.
3. Patients undergoing pancreatic resection—prophylactic.

The drug was administered randomly and double blind. The study was terminated by withdrawal of the pharmaceutical house from distribution of trasylol. The code was broken for this report. The initial dose was 200,000 followed by 200,000 q.4.h. for three days. In the prophylactic studies, the drug was started the day before surgery and administered three days after. The results are given in Table IV. No differences can be discerned between the control and trasylol data.

While my patient group differs from Trapnell's, the protocols are similar. The data convince me that trasylol therapy is without effect on the course of human pancreatitis.

Summary

The clinical and experimental reports concerning use of trasylol have been reviewed and despite a recent glowing report of its efficacy, trasylol revisited is found wanting.

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Amelioration of Streptozotocin-Induced Diabetes in Mice by the Implantation of Pancreatic Islets in Diffusion Chambers*

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Abstract

The effects of syngeneic pancreatic islets enclosed in membrane diffusion chambers were investigated after intraperitoneal implantation into streptozotocin-diabetic CBA mice. The diabetic state was significantly ameliorated after implantation as judged by plasma insulin levels and glycosuria determinations. The improvement seen in mice receiving islets in chambers persisted more than two weeks and was comparable to that of control mice directly implanted intraperitoneally with islets. The best implant function was obtained using minimal volume "nucleopore" membrane chambers (thickness 10 microns, 1 million 0.45 micron pores per square centimeter).

Over the past ten years it has become evident that the administration of daily doses of insulin does not afford sufficiently precise regulation of the diabetic state. With such therapy, the secondary complications of diabetes are not prevented and the morbidity and mortality from blindness, as well as renal, cerebral, and cardiovascular disease, are unaffected. Currently, attention is being focused on the possibility of using pancreatic transplantation as a cure for diabetes. Surgical transplantation of the whole organ, both in dogs and man, has posed numerous technical and postoperative problems (1), motivating several groups to explore the use of isolated pancreatic islets (2, 3, 4). Such transplanted islets have been shown to function in syngeneic hosts and can forestall the development of symptomatic diabetes. Recently, the regression of glomerular lesions, in conjunction with sustained improvement of glucose levels, has been demonstrated in Lewis rats following treatment with isogeneic islets (5).

Allogeneic islets are rejected by the recipient's immune system just as are other tissue allografts, so that immune suppression with agents such as antilymphocyte globulin is necessary for the continued function of the graft (6). Alternatively allografted islets have been implanted into an "immunologically privileged" site such as the testis to minimize the cellular component of rejection. Some of these implants have remained functional for long periods;

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however these sites do not afford total protection of grafted tissues. The use of cell-tight membrane diffusion chambers to forestall immunologic rejection was investigated over 20 years ago by Algire (7). The recent availability of thinner, more porous membrane materials prompted us to examine survival and function of islets placed in such diffusion chambers inserted intraperitoneally into streptozotocin diabetic mice and to compare the improvement in the diabetic state with that observed after direct injection of islets.

Materials and Methods

Streptozotocin-Diabetic Mice: Diabetes was induced in male 30 gram CBA mice (Jackson Laboratories, Bar Harbor, Maine) by intravenous injection of streptozotocin in buffered citrate saline (pH 4.5) 20 mg per mouse. Glycosuria was monitored using Lilly TesTape®.

Grafting of Islets: Pancreatic islets were prepared from adult CBA mice by a modification of the method of Lacy and Kostianovsky (8). Minced pancreatic tissue was incubated for 30 minutes at 37°C in 2 ml of Minimal Eagle's Medium (MEM, Microbiological Associates, Bethesda, Md.) containing 5 mg type IV collagenase (Worthington Biochemical, Freehold, N. J.). When digestion was complete a few crystals of DNAase were added and incubation continued for one minute. Fifteen ml MEM was added and the diluted material was strained to remove large particles. The suspension was filtered twice through a 70-micron mesh stainless steel screen: material retained on the sieve was rinsed several times with MEM and washings were collected and centrifuged. The supernatant was discarded and the pellet resuspended and washed. The yield of islets was 800–1000 per mouse; the islet preparation examined under the light microscope contained some acinar debris but no organized excrone elements.

To assess viability, islets were incubated for ten minutes with 0.12 M fluorescein diacetate in MEM, washed twice and examined with a Zeiss ultraviolet microscope equipped with an Osram HB 200 mercury lamp and a Ploem vertical illuminator. Viable islets showed strong green fluorescence; acinar debris did not fluoresce.

Chambers were constructed from nucleopore membranes with a pore size of 0.45 microns and a pore density of 10^6 per square cm. Two membranes were heat sealed together to form a small envelope about 13 mm wide by 20 mm long. This was filled with .4–.7 mls of islet cell suspension but quickly collapsed almost completely as medium filtered out.

Host mice receiving chambers were anesthetized with ether. Diffusion chambers containing islets (vide infra) were inserted into the peritoneal cavity through a midline, three cm incision and were sutured in place to minimize the possibility of intestinal obstruction or volvulus. Glycosuria was measured every two days using TesTape (Lilly).

Islets were suspended in .7% agarose at 40°C. This suspension was injected under the kidney capsule via a 25-gauge needle using an anterior abdominal incision as previously described.

Plasma Insulin Radioimmunoassay: Blood was collected from the eye sinus of the mice in heparinized capillary tubes. 0.05 ml of plasma was mixed with 0.05 ml of insulin-binding reagent (Amersham-Searle) dissolved in deionized water. 0.05 ml phosphate buffer pH (7.9) was added and the mixture incubated for six hours at 4 C. Radioactively labeled I^{125} Insulin was then added and an additional 18-hour incubation was performed. The precipitate was collected by filtration and washed. I^{125} radioactivity was determined using a gamma well counter, and assayed against standard samples of human insulin.

In vitro insulin production: Insulin production by islets in vitro was assessed in replicate short-term cultures containing different numbers of islets, in medium containing either 0 mg or 30 mg% glucose. The time course of insulin production was plotted over a one-hour period.

Results

The severity and reversibility of Streptozotocin-induced diabetes was followed in groups of 10 mice given 12, 18 or 24 mg. of the drug. Insulin levels in random serum samples of unfasted normal mice ranged between 25–50 milliunits/ml as measured against a human insulin standard (Table I). Decline in insulin levels was observed within four hours after injection of streptozotocin. By the third day serum insulin levels approached zero in those mice receiving the highest dose. Seven of the 10 animals administered 24 mg streptozotocin showed glycosuria within four hours, associated with marked polyuria and progressive wasting; only two of these mice survived to day 10 postinjection. Mice receiving 12 mg streptozotocin showed a fall in insulin activity to 10% of normal, yet no more than trace glycosuria developed. Glycosuria in all mice was followed for two weeks and no spontaneous remission was observed.

Islets incubated in vitro showed a rise in insulin levels in the supernatant during the hour of incubation. The rise was greater in islets incubated with medium containing glucose (Table II).

The in vivo viability of islets was assessed by injecting 200, 2,000 and 10,000 islets intraperitoneally into streptozotocin diabetic mice (Table III). An initial rise in plasma insulin levels was noted two hours posttransplant. At 24 hours mice receiving 10,000 islets had an increase in plasma insulin to three times

TABLE I

Random Insulin Levels Were Measured in Adult Male CBA Mice. Mice Were Then Injected with Variable Doses of Streptozotocin and the Decline in Insulin Levels Was Followed over a Three-day Period.

Dose of Streptozotocin	Control Insulin Levels	1 Hour	Day 3
4 mg/100 grams	45.9	26.3	6.0
6 mg/100 grams	43.5	21.8	5.4
8 mg/100 grams	24.4	18.7	0

TABLE II

Two Concentrations of Islets Were Incubated for One Hour with or without Glucose Present. Insulin Concentration in the Supernatant Was Determined at 15 Min Intervals.

In Vitro Insulin Production		Time	Microunits Ins/Ml
Islets/Ml	% Glucose		
460	30 mg%	15 min	170.79
		30 min	197.96
		45 min	331.88
		60 min	504.61
460	0 mg%	15 min	250.36
		30 min	267.83
		45 min	448.83
		60 min	262.01
92	30 mg%	15 min	52.28
		30 min	57.80
		45 min	61.60
		60 min	61.60
92	0 mg%	15 min	40.00
		30 min	62.20
		45 min	57.40
		60 min	61.40

TABLE III

Mice Were Transplanted with Islets Injected Intraperitoneally. Insulin Levels Were Compared before and after Transplant.

Insulin Levels after Intraperitoneal Islets					
Microunits Insulin 1 Ml Plasma			Pre Transplant	2 Hrs Post Transplant	24 Hrs Post Transplant
Group		4 Hrs Post strep			
I	500 islets	7.2 ± 3.4	4.5 ± 1.2	12.0 ± 2.7	20.4 ± 1.0
II	2,000 islets	15.3 ± 4.7	14.1 ± 3.8	11.5 ± 3.8	18.7 ± 4.9
III	10,000 islets	11.1 ± 3.6	6.0 ± 2.9	17.2 ± 6.1	27.1 ± 2.7

the original levels. Immediately after transplant glycosuria fell from +3 or +4 to a range of 0 – +2. The diminished urinary glucose persisted for at least four days in the transplanted group while no change was observed in the group of nongrafted diabetic mice.

The efficiency of chamber-enclosed islets was compared to intraperitoneally injected islets in improving serum insulin levels and reducing glycosuria (Tables IV and V). Groups of five diabetic mice received either no treatment, 10,000 islets or the same number of islets sealed in diffusion chambers. An increase in plasma insulin levels was noted 90 minutes after transplantation in the injected group; the rise of the plasma insulin levels was delayed in the chamber transplanted group. After one day the insulin levels in both the directly injected and chamber-implanted groups were equivalently higher than

TABLE IV

Insulin Levels Were Compared in Normal Mice. Streptozotocin Diabetic Mice and Mice Transplanted with Islets Placed in Chambers or Islets Injected Intraperitoneally (IP)

Group	4.5 Hrs P. Strep	Day 3 P. Strep	1.5 Hrs P. Graft	Day 1 P. Graft	Day 3 P. Graft	Day 4 P. Graft	Day 14 P. Graft
A. microunits insulin/ml normal-not diab	20.6 ± 4.3	N.D.	13.0 ± 1.6	21.0 ± 2.0	22.8 ± 2.1	13.0 ± 1.3	26.2 ± 5.4
B. insulin glycosuria strep diab	18.4 ± 2.6	11.2 ± 9.7	8.0 ± 1.1	11.2 ± 1.7	12.2 ± 6.6	8.2 ± 1.7	16.4 ± 2.5
C. insulin glycosuria 10,000 islets IP	13.0 ± 2.3	9.0 ± 1.1	28.8 ± 3.9	10.0 ± 7.9	13.8 ± 1.7	8.0 ± 8.6	13.12 ± 1.6
D. insulin glycosuria sealed islet in chambers	13.2 ± 2.8	9.3 ± 4.5	9.8 ± 1.2	14.6 ± .3	9.8 ± 1.1	14.8 ± 5.4	12.30 ± 1.4

TABLE V

Groups are the Same as in Table IV. Glycosuria Was Measured Using Lilly TesTape® before and after Transplant. Numbers Represent Average Glycosuria (on a Scale of 0 to 4) of 5 Mice.

Group	4.5 Hrs P. Strep	Day 3 P. Strep	Day 1 P. Trans	Day 3 P. Trans	Day 4 P. Trans	Day 7 P. Trans	Day 11 P. Trans	Day 14 P. Trans	Day 17 P. Trans
B	3.0	3.6	2.2	2.8	3.0	3.5	3.0	2	2
C	3.0	4.0	1.6	1.5	1.5	1.5	1.2	1	1.5
D	2.2	2.7	2.7	1.5	1.5	1.7	1.5	1.5	1.2

TABLE VI

Mice Were Treated with Intraperitoneal Islets or Islets Embedded in Agarose Matrix. Survival Time and Glycosuria Were Compared in Treated versus Untreated Animals.

Number of Mice	Treatment	Median Survival After Streptozotocin	Diabetes Amerliorated
13	None	15.2 ± 2.3	0
15	Intraperitoneal islets	3.4	11
8	Agarose	2.7	7

untransplanted controls. However, by day 4 insulin levels in the transplanted groups had declined to the levels of the control groups. The improvement in glycosuria nevertheless persisted for more than two weeks.

The contents of the implanted diffusion chamber were examined by phase microscopy after removal from normal or streptozotocin diabetic mice. Islet architecture was not maintained; after seven days in the chamber only single cells, many of which resembled β-cells were present. These cells were viable as judged by their fluorescence after incubation with fluorescein diacetate.

Mean survival time after induction of diabetes was also compared in untreated mice, mice injected with islets intraperitoneally, and mice injected with a suspension of islets in agarose. Average survival times in the untreated group were approximately half of the group treated with agarose suspended islets and less than half of the survival time in the intraperitoneally injected group. 73% of the intraperitoneally injected and 85% of the agarose injected showed a decrease in glycosuria (Table VI).

Discussion

Using the streptozotocin model of diabetes we have shown that mouse pancreatic islets may survive and produce insulin both when injected intraperitoneally and when enclosed in diffusion chambers. Although Ballinger and Lacy (2) implied that vascularization may be necessary to ensure function, our preliminary data indicate that survival may be obtained even without a direct blood supply. Reemtsma (9) has demonstrated production and release of insulin for 3 days by fish islets in millipore diffusion chambers implanted into diabetic rats; using Cupraphan chambers Weber and Reemtsma (10) obtained week-long survival and function of some piscine grafts.

The diabetes, as measured by glycosuria or increased mouse survival was only partially improved by the grafts. Beyond the fourth day posttransplant, insulin levels were only insignificantly higher in the transplanted group. Scharp (11), obtained similar result with hepatic injections of islets in unrelated monkeys; there is reduction of polyphagia, polydypsia, glycosuria and hyperglycemia with only a minimal change, both in basal and glucose tolerance test levels, in peripheral insulin.

The incomplete remission of the diabetic state may be due to an insufficient number or size (which is related to insulin content) of the transplanted islets. Kramp et al (12) obtained sustained remission after transplanting hyperplastic islets from ObOb (congenitally obese C57BL) mice. In addition, the location of the islets may not have been optimal. Kamp et al (13) has demonstrated that injection of islets directly into the portal vein of the liver was more effective in decreasing glycosuria and increasing weight than intraperitoneal implantation. The limitation on continued survival in chambers may have been due to an accumulation of toxic metabolic products or the maldistribution of islets within the chambers. Blockage of membrane pores by endothelial or fibroblastic growth may curtail diffusion of necessary nutrients.

If these problems can be overcome the nucleopore membranes represent a distinct advantage over membranes used to date. The thickness is 10 microns as compared to a minimum of 25 microns for other commercially available materials. The number of pores is substantially greater per unit area in nucleopore membranes; a pore density of 1 × 10 (6) represents 12% of the total surface area of the filter. By placing the tissue in such cell-tight chambers, permeability to entering nutrients and secreted insulin may be maintained while contact with immunocompetent lymphocytes will be prevented. The use of diffusion chambers as immunologically privileged sites thus represents an approach to allogeneic and xenogeneic grafting of islets without immunosupression.

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The Use of Gastric Pouches in Gastric Physiology: I. Techniques in the Preparation of Gastric Pouches*

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> *Our first problem consisted, therefore, in the working out of a method. It was necessary to know how the reagents were poured out upon the food brought into the digestive factory. To accomplish this in an ideal manner required the fulfillment of many and difficult conditions. Thus it was necessary to be able to obtain the reagents* at all times, *otherwise important facts might escape us. They must be collect in* absolutely pure condition, *if we were to determine how their compositions varied, and also in* accurately measurable quantities. *Lastly, it was necessary that the* digestive canal should perform its functions normally, *and that the* animal *under experiment should be in perfect health* (*Pavlov, 1910*).

Dr. Ralph Colp was an outstanding clinical surgeon, yet recognized the need for investigative surgery. He encouraged young surgeons to pursue a career in research and was instrumental in guiding members of the surgical staff in that direction. One of his main interests was gastrointestinal surgery. For that reason we present the following review for investigators interested in gastric physiology.

The remarks of Pavlov (1) summarize the requirements for the study of gastric physiology today as well as they did when he originally enunciated them. Although physiologists frequently turn to in vitro preparations, current sophisticated technology allows much information to be obtained in conscious animals. The need for studies utilizing gastric pouches, therefore, increases rather than suffers from disuse. Each animal can be used repeatedly under a variety of conditions, will serve as its own control, yet will allow accurate measurements of pure uncontaminated secretion without altering the essential functions to be studied. The use of chronic experiments enables repeated experiments in the same animals, sparing the total number of animals required. For many reasons the dog is most commonly chosen for pouch studies, but the techniques can be adapted to most other animals.

There is no perfect preparation to study all modalities of gastric secretion. The type of pouch used depends entirely on the questions asked. For this reason, a variety of different pouches is used.

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Anatomy

The anatomy described hereunder is only a brief summary of the anatomy of the canine stomach. A complete understanding is important, however, in order to construct correctly the various types of pouches. For more detailed description and illustrations the reader is referred to one of the many excellent standard textbooks of canine anatomy.

The stomach is relatively large, with an average capacity of 3 liters, in a dog of average size (100–200 ml/Kg body weight). It consists of a proximal acid and pepsin-secreting area and a distal nonsecreting area. The oxyntic gland area (corpus) is large and rounded, while the pyloric gland area (antrum) is small and cylindrical. The corpus commences at a point where the esophagus enters the stomach, and is large and rounded, convex to the left; its greater curvature is nearly four times as long as the lesser curvature. The upper part of the lesser curvature is nearly straight and vertical, but the lower part forms a deep, narrow angle (incisura angularis) because the pyloric part is directed sharply forward and upward. Transition from the antrum to the duodenum is well demarcated by the pyloric sphincter.

Whereas the fundus is responsible for the secretion of acid and pepsin, no acid is secreted in the antrum; the hormone gastrin is elaborated here. Since pure fundic or antral pouches may be constructed, the differences between the two should be recognized. The demarcation between antrum and corpus is relatively easily defined in the dog. Several points facilitate the easy identification of the dividing line between corpus and antrum: 1) the serosa of the antrum is generally paler than that of the corpus; 2) the serosal terminal vessels of the corpus have a "kinky," somewhat granular appearance, when compared to those of the antrum, and 3) the antral mucosa is relatively pale, firm, and flat, whereas the mucosa of the corpus is more suffused with a purplish tint, is softer, and thicker with more prominent rugae. The transitional zone of mucosa between corpus and antrum generally extends for no more than 1.5 cm, and occurs at the incisura angularis along the lesser curvature. A line projected from the incisura angulars, perpendicular to the proximal lesser curvature, will generally fall across the junction between corpus and antrum.

Arterial blood supply of the stomch is derived from the celiac artery, which divides into the left gastric, splenic, and hepatic arteries. The left gastric artery is proportionately larger than in man and approaches the lesser curvature of the stomach from the cardia, ultimately anastomosing with the smaller right gastric artery, a branch of the hepatic artery. After giving off several large, short gastric branches to the upper third of the gastric curvature directly, the splenic artery forms a large branch, the left gastroepiploic artery, which runs along the greater curvature; here it anastomoses with the right gastroepiploic artery, which runs along the greater curvature; here it anastomoses with the right gastroepiploic artery (a branch of the hepatic). The short gastric vessels are larger than in man and supply one-third of the greater curvature. The venous drainage follows a similar pattern, all the tributaries ultimately entering the portal venous system.

Afferent and preganglionic parasympathetic efferent fibers to the abdominal viscera are contained in the vagus nerves. In the chest, the left and right vagus nerves each divide into a ventral and dorsal division at the level of the hilum of the lung. The ventral branch of the left vagus is usually larger than the dorsal branch, and the dorsal branch of the right vagus is usually larger than the ventral branch. The ventral division of the right vagus passes almost horizontally to unite with the ventral division of the left vagus and thus form the anterior vagus nerve, more or less at the level of the hilum. The dorsal division of the left vagus passes obliquely along the left side of the esophagus, where it unites with the dorsal division of the right vagus to form the posterior vagus nerve a very short distance above the esophageal hiatus. Examination of the vagus nerves in the lower chest usually reveals three trunks—the anterior vagus and the two divisions of the posterior vagus. Immediately upon entering the abdomen, the anterior vagus gives off its hepatic branches and then traverses along the lesser curvature of the stomach as the Nerve of Latarjet, which gives off the gastric branches obliquely from the lesser curvature. The posterior vagus, upon entering the abdomen, gives off a celiac branch, and then also traverses the lesser curvature in a similar to the anterior nerve fashion. Thus, each vagus trunk in its passage down the stomach bears a

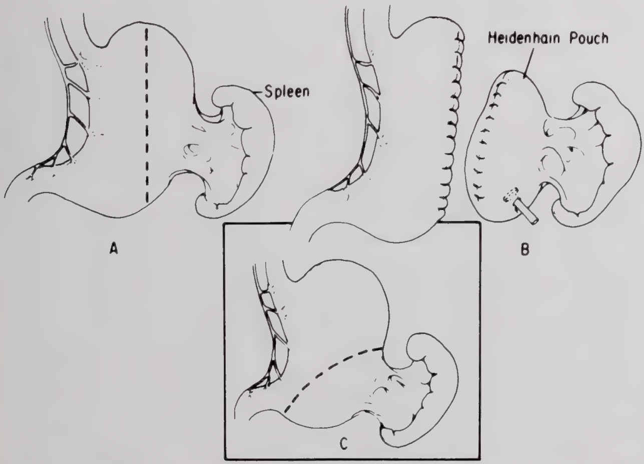

FIG. 1. Classic Heidenhain pouch: (a) shows line of transection and (b) completed pouch. (c) shows commonly used but incorrect method of constructing pouch—this incorporates some antrum in the pouch.

constant relation to the lesser but not to the greater curvature, and gives off branches which enter the muscularis about one-third of the distance between the lesser and greater curvatures.

The significance of the distribution of the vagi will be readily appreciated when preparing vagally innervated pouches. Four practical points bear mentioning: 1) Any procedure involving division of the vessels along the lesser curvature results in at least a partial denervation of the stomach; 2) It is possible to selectively divide or retain the vagal innervation to the gastric corpus or antrum alone; 3) It is also possible to selectively divide or retain the vagal innervation to the gastric corpus or antrum alone; 4) It is also possible to selectively divide the main vagal trunks, the hepatic branches, the celiac branch, or only the gastric fibers to either the fundus or antrum (or both); and 5) Since neither the anterior nor the posterior vagal trunks proximal to the hepatic and celiac branches is present in the abdominal cavity, identification and exposure of these trunks usually entail opening the pleural cavity. This should be borne in mind when isolating the anterior and posterior trunks. A respiratory should be available during any such procedure.

The sympathetic nerve supply arises from the celiac plexus, the nerve fibers and ganglia forming a dense network around the celiac artery and its primary branches.

Techniques of Pouch Construction

Denervated Pouches

Heidenhain (fundic) pouch (Fig. 1) (2, 3): This is probably the most widely used gastric pouch, and many investigators apply the term Heidenhain pouch to any type of fundic pouch deprived of its vagal innervation. Because of the distribution of the secondary vagal branches, a fundic pouch in which the anterior and posterior walls of the stomach are completely divided in its preparation will be devoid of vagal innervation.

The stomach and spleen are delivered into the wound. Babcock forceps are placed at multiple points along the greater curvature so that the stomach can be stretched out, allowing a line of incision for the pouch to be selected. At the lower end of the proposed line of transection, the greater curvature should be cleared for one to two centimeters, by dividing the gastric branches of the right gastroepiploic artery and the gastroepiploic artery itself. This gap should be extended into the adjacent omentum to avoid tearing the omental vessels during subsequent manipulation. The uppermost margin of the proposed line of transection must be above the vasa brevia, and this portion of the greater curvature of the stomach should also be cleared for one to two centimeters; this is a simple matter, as the gastrocolic ligament is relatively avascular at this point. The stomach should be kept stretched and flattened, so that long, noncrushing intestinal clamps may be placed along the long axis of the stomach at the proposed line of transection. Many investigators employ a stapling clamp to control bleeding and leakage of gastric content, dividing the stomach completely between two rows of staples. However, a satisfactory technique can be achieved with intestinal clamps alone. Before commencing the suturing, the uppermost two or three staples on the pouch side are removed to allow introduction of the gastric cannula, which is then brought out through the anterior wall in the most dependent part of the pouch. A simple technique for performing this is described later. The main stomach and pouch are each closed in two layers. Care must be taken in the closure of the main stomach not to produce an area of constriction in the remaining stomach between the line of closure and the lesser curvature at the reentrance angle. Should this be a problem, the defect in the stomach wall may be closed transversely. The pouch derives its blood supply solely from the short gastric and left gastroepiploic branches of the splenic artery, which should be carefully preserved. A purse-string suture is placed in the anterior wall of the pouch around the cannula to hold it in place. A double sheet of omentum is then wrapped around the pouch, and the cannula led through it, before the cannula is pulled through the anterior abdominal wall.

It is desirable to construct as large a pouch as possible to obtain a preparation which is sensitive in its response to stimuli. Since there is negligible resting secretion from a Heidenhain pouch in a fasted animal, it is useful for testing the effects of various gastric secretory stimulants. Gastric secretory inhibitors may also be tested when the pouch is secreting in response to a given stimulus. There are two inherent advantages in the utilization of denervated fundic pouches; 1) pouch output is more uniform since it is freed from vagally mediated factors (hunger, anxiety, fear) that affect the rate of gastric secretion; and 2) losses of fluids and electrolytes are less than with innervated pouches which secrete a larger volume of gastric juice daily.

Bickel pouch: The Bickel pouch (4) is a vagotomized and completely neurectomized pouch. The vagotomy is achieved by transecting the entire wall of the stomach as for a Heidenhain pouch, while the sympathetic dennervation of this pouch is achieved by isolating the blood vessels and tearing the fine nerves across with a blunt instrument. However, this is a delicate procedure which requires considerable skill in order not to damage the vessels. There is a strong possibility of missing some fibers, thus failing to effect a complete sympathectomy. In order to achieve complete denervation, a celiac ganglionectomy may be added to this procedure as a means of denervation, but this involves extensive denervation of the abdominal viscera (intestinal motor disturbances have been reported following such operations). Perhaps the simplest technique is the Gregory (5) modification. A Heidenhain pouch is initially created and the spleen is removed after ligating the blood vessels close to the hilum so that the vascular communications between the splenic blood vessels and the pouch are not interrupted. These blood vessels are then traced toward their origins and the connective tissue cleaned off in layers for about two centimeters.

In contradistinction to Bickel's original method, Gregory's method is considerably easier because of the large size of the blood vessels at their origin; here the nerves are collected into a few large bundles which can be easily removed. These pouches respond to feeding more rapidly than Heidenhain pouches which have a much tardier response.

Total gastric pouch (denervated): In such a pouch, the major stimuli effecting secretion originate in the intestine (6). To facilitate exposure to the lower end of the esophagus, and to simplify the subsequent esophagoduodenal anastomosis, this procedure is best approached through a long left oblique incision. The lower esophagus is divided just above the cardioesophageal junction, and the upper end of the stomach closed. It is tempting to retain a small cuff of stomach in this region because of the lower mortality and ease of subsequent anastomosis to the duodenum, but the acid secretion from this small cuff of gastric mucosa may affect the subsequent experimental results. At the pyloroduodenal junction, the hepatoduodenal ligament is divided in order to promote greater mobility of the duodenum, and the stomach is divided at the level of the pylorus. The cannula is now introduced through the pyloric opening, and is brought out through the mid-anterior wall of the stomach in a dependent position proximal to the antrum. In the past, the cannula was usually placed near the pyloric end of the stomach, but it has been convincingly demonstrated (7) that the mechanical presence of a cannula may result in a continuous release of endogenous gastrin in these preparations. It is, therefore, preferable to bring the cannula out through the anterior wall proximal to the antrum. Continuity is restored by either an esophagoduodenal or esophagojejunal anastomosis. This pouch is particularly useful for studying the action of stimulatory or inhibitory substances of intestinal origin on gastric secretion.

Denervated antral pouches: In order to study accurately the release of gastrin by the antrum and mechanisms of its inhibition, it is necessary to prepare pure antral pouches completely devoid of any oxyntic glands. It is possible to delineate the junction between corpus and antrum by the colorimetric method of Moe et al. (8). Prior to construction of the antral pouch (with the animal still under anesthesia), a small dose of histamine is injected intravenously to stimulate gastric secretion. The anterior wall of the stomach is incised at approximately the corpus-antrum border (or a gastrotomy is performed) and hemostasis secured. A thin layer of moist white gauze is placed on the gastric mucosa where the corpus-antrum border is anticipated, to conform closely to mucosal contours. Ten ml 1% toluidine blue in 5% dextrose-water is injected intravenously over a period of two minutes. Within three to five minutes a blue color develops over the corpus while the antum remains unstained. Neutral red may also be used, but the color may be obscured by blood. Dilute fluorescein has also been advocated, but this technique requires a darkened operating room during use of an ultraviolet light to detect fluorescence. The layer of gauze serves three purposes: It prevents the dye color from being obscured by the color of gastric mucosa; the appearance of the dye is noted more rapidly; it prevents gastric juice containing dye from spilling over from the corpus into the antrum. When using this method it is always possible to see a clearcut border between corpus and antrum. Litmus paper may also be used but, in general, it does not demonstrate the antrum-corpus junction as clearly as the colorimetric method. One to two centimeters of antrum-corpus junction should be removed at the transitional zone to exclude any possibility of retained parietal cells; a small piece of mucosa from the antral side is then removed for histology to confirm the absence of oxyntic glands.

The antrum is closed proximally in two layers. All remaining vagal connections are severed by clearing the lesser curvature of the antrum to just beyond the pyloroduodenal junction. At the pyloroduodenal junction, the greater curvature is cleared for 1–2 cm, *care being taken to remain within the vascular arcade*, as the blood supply to the antral pouch will be entirely derived from the right gastroepiploic artery. The duodenum is transected just distal to the pyloric sphincter and gastrointestinal continuity restored by either a gastroduodenal anastomosis or by a gastrojejunostomy with closure of the duodenal stump. The antral pouch is exteriorized as a mucocutaneous fistula to the right of the midline; a cannula is not usually introduced into the antral pouch since it may result in the continuous release of endogenous gastrin. An ellipse of skin is excised and the stoma of the pouch everted, creating a stoma similar to an ileostomy spout. Four silk sutures are inserted circumferentially taking the following tissues in order: skin, peritoneum, seromuscular layer of antrum 1.5 cm proximal to the open end, and through the whole wall of the antrum at its cut end. After tying these sutures, a further four mucocutaneous sutures are applied between. It is advantageous to retain the pyloric sphincter so that subsequent irrigation of the antral pouch may be achieved by inserting a Foley catheter, which can be retained simply by inflating the balloon. The anterior wall of the pouch is approximated to the parietal peritoneum by two or three sutures so that the pouch will be in a horizontal position when the dog is standing; any irrigating fluid will not run out immediately and can be easily controlled. It may be necessary to insert a cannula into the antral pouch, but these are usually extruded by the pouch and seldom remain in place for a few months. Leakage around the cannula in antral pouches is a common occurrence.

A fundic pouch is frequently constructed in the same animal to act as an indicator pouch. Both stimulatory and inhibitory mechanisms of the antrum can be studied by constructing two antral pouches.

Transplanted pouches: Only when a gastric pouch is completely devoid of all nervous innervation can unequivocal proof be obtained of the existence of a humoral mechanism of gastric secretion. This can be achieved by an autotransplanted or homotransplanted pouch. In the past an autotransplanted pouch was prepared in two stages (9). At the first stage, a Heidenhain pouch, supplied by a single artery and vein, is constructed and is brought through a stab wound at or adjacent to a mammary gland (preferably using recently whelped bitches). By blunt dissection in the region of the mammary gland (because of its good blood supply), a cavity is created into which the pouch is placed. At a second operation, the vascular pedicle is stripped of its nerves and connective tissue and the vessels pointed with phenol or iodine in ethanol, resulting in subsequent fibrosis. This produces a gradual decrease of the blood supply from the pedicle, causing the pouch to rely increasingly on the new vessels growing in from the surrounding tissues. At a third operation, if clamping the pedicle with the fingers fails to cause blanching of the mucosa of the pouch stoma, the pedicle is transected and the stump connected with the pouch covered with peritoneum to prevent formation of vascular adhesions which may carry fibers to the pouch. These pouches are less responsive than Heidenhain pouches to secretagogues because of their poor blood supply; they are also greatly influenced by vasomotor changes in the region in which they are situated. A far superior technique is the use of direct vascular anastomoses to achieve vascularization of the pouch (10–12).

Following splenectomy, a Heidenhain pouch is prepared and the splenic artery and vein are mobilized down to the tail of the pancreas (more extensive mobilization frequently results in pancreatic necrosis). Vascular clamps are applied to the splenic vessels well away from the pouch, and the vessels are ligated and divided proximal to the clamps. In adult dogs, the splenic arteries and veins are usually 2.0–3.0 mm and 3.0–5.0 mm external diameter, respectively. The vessels are individually flushed with heparin-saline solution. The recipient site is prepared for receiving the transplant. Two sites are commonly used: 1) The neck. A cavity is created by blunt dissection and the omocervical trunk and external jugular vein are mobilized; vascular clamps are applied proximally, and the vessels divided after distal ligation; 2) The abdomen. Left nephrectomy is performed and the renal artery and vein similarly prepared.

The appropriate vessels are then anastomosed by direct suturing with 6–0 vascular silk or by a vascular clamp. The small vessel anastomosis instrument devised by Nakayama et al. (13) is ideal for this purpose. In each case the vessel is connected to a ring and fixed in position on the pins of the ring without injuring the intima and allowing for even distribution of the vessel wall about the ring. After each ring has been applied, the corresponding arteries are aligned to prevent torsion, and the two ring holder clamps pressed tightly together with pliers. The clamps are removed to confirm the flow of blood through the pouch; this procedure is repeated for the veins. The abdominal incision is closed after the cannula is placed in position.

The use of direct vascular anastomosis avoids staged procedures, completely denervates the pouch, has a low incidence of vascular thrombosis and histologic changes in the mucosa, and provides for good acid secretion due to the adequate blood supply. Furthermore, this technique is also suitable for studies involving homotransplantation of the stomach.

Transplantation of the isolated antrum can also be performed in this manner, but, because the right gastroepiploic vessels may be not more than 1 mm diameter, is frequently complicated by thrombosis of the vessels.

Innervated Pouches

Gastric fistula: The simplicity of this preparation is its chief advantage; a cannula is merely placed in a dependent part of the stomach. It thus allows minimal alteration to the alimentary canal while preserving vagal innervation.

There are, however, several disadvantages: Incomplete collection of gastric juice; contamination by duodenal contents; contamination by saliva; and it does not permit study of secretion during a meal. Concomitant esophagostomy (14) prevents contamination by saliva and allows the response to sham-feeding to be studied. A gastric fistula may be created in the gastric remnant after construction of a Heidenhain pouch; the effect of a secretory stimulant can thus be studied in both vagally innervated and denervated preparation in the same animal.

Total gastric pouch (*Fig. 2*) (*15*): At the outset of the operation the endotracheal tube should be connected to a respirator because dissection of the esophagus and major vagal trunks invariably results in opening of the pleural cavity. A long left oblique incision is made, traction is placed on the stomach and the phrenicoesophageal ligament is divided. The lower esophagus is freed by blunt dissection and a soft tube is passed around it. The vagus nerves are identified and gently retracted away from the esophagus by umbilical tape. Two noncrushing clamps are placed on the esophagus and stomach, respectively, carefully avoiding damage to the vagus nerves. The esophagus is divided between the clamps. The lower end of the stomach is then divided at the level of the pylorus after mobilizing the vessels on the greater and lesser curvatures at this point. A cannula is introduced into the stomach at a dependent position and both cardiac and pyloric ends of the stomach are closed in two layers. Continuity is restored by an esophagoduodenal or esophagojejunal anastomosis. It is possible to subsequently convert this pouch into a denervated total gastric pouch by simply dividing the vagus nerves.

These animals require a considerable amount of care and dietary supplement. Because of the large loss of gastric secretion, fluid and electrolyte

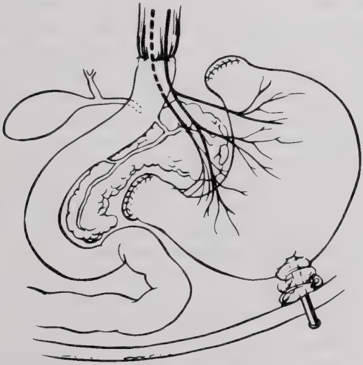

FIG. 2. Vagally innervated total gastric pouch.

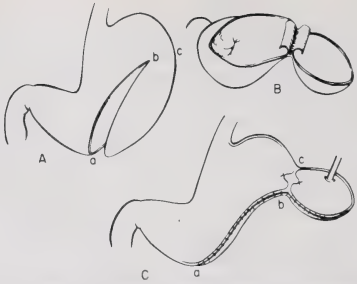

FIG. 3. Classic Pavlov pouch: (a) forceps inserted through small incision grasping mucosa at x and y; (b) mucosa of pouch evaginated; (c) incision of mucosa.

depletion may rapidly develop unless the gastric juice is returned and saline infusions administered daily.

Innervated fundic pouches (*Pavlov pouches*) (*Fig. 3*): Although innervated fundic pouches are generally referred to as Pavlov pouches, the classic Pavlov pouch (1) retains very little vagal innervation. The gastroepiploic vessels and greater omentum are divided at the lower limit of the pouch. Two noncrushing clamps are placed along the longitudinal axis of the stomach, extending about two-thirds of the length of the stomach (along ab in Fig. 3A). Both anterior and posterior walls of the stomach are completely divided between the clamps, i.e., from a to b. The clamp on the pouch side is removed and hemostasis secured. The proposed pouch is now pulled back to form an everted flap hinged along bc. An incision is made through the mucosa of the anterior and posterior walls between b and c, so that the pouch remains attached to the stomach by the seromuscular layers only (Fig. 3B). The mucosa of the anterior and posterior walls of the stomach between b and c are sutured together and the suture continued along ab, which is then reinforced with seromuscular layer. This technique is repeated for the pouch so that a double mucosal septum is created at bc (Fig. 3C). Before completing closure of the pouch, a cannula is introduced into the pouch.

A number of modifications of the Pavlov pouch have been described. They all attempt to simplify the construction of the pouch while reducing the rate of fistulization and retaining maximal vagal integrity. The interested reader is referred to our earlier publication (16) for further details. We shall only describe three of the currently used modifications.

A. Thomas modification (*Fig. 4*) (*17*): After selecting the site for preparation of the pouch a 2 cm incision is made in the center of the pouch area just anterior to the greater curvature. Two Allis clamps are inserted through this incision to grasp the mucosa at points x and y. Gentle traction is placed on these forceps until the entire pouch mucosa is everted through the incision. The mucosal surface of the pouch area now appears as a circular rosette which

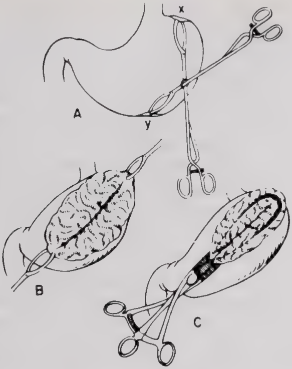

FIG. 4. Thomas modification of Pavlov pouch: (a) forceps inserted through small incision grasping mucosa at x and y; (b) mucosa of pouch evaginated; (c) incision of mucosa.

is converted into an oblong shape by pulling the forceps in opposite directions. A Lane twin-gastroenterostomy clamp or a Roosevelt 3-jawed gastroenterostomy clamp is applied so that the center jaw or jaws lie between the ridges of mucosa. An incision is made through mucosa only along the crest of each mucosal ridge completely encircling the pouch area. Mucosal flaps are formed by undermining the mucosa for 1 cm along each side of the incision. We find it preferable to excise a ribbon of mucosa about 0.5–1.0 cm wide from the crest of the rosette. The two inner flaps of mucosa (which form the anterior and posterior mucosal surfaces of the main stomach) are sutured together using an inverting Connell suture. Fistula formation can be reduced by suturing the submucosa of the anterior wall to the submucosa of the posterior wall with a continuous suture. The outer mucosal layers (which form the anterior and posterior mucosal surfaces of the pouch) are approximated with a Connell suture, thus creating a double mucosal septum between pouch and main stomach. The mucosa of the pouch is now returned to its original position, a cannula inserted into a dependent position and the original small incision in the center of the pouch closed. This pouch retains maximal vagal innervation, is simple to construct, but is subject to fistula formation.

B. *Perry modification* (*Fig. 5*) (*18*): This method depends on the careful nontraumatic dissection of the anterior vagal trunk and its branches. Its major advantage over the previous modifications is the prevention of fistula formation between the pouch and main stomach.

Although a thoracic approach was orginally advocated by Perry et al, the operation can be carried out transabdominally without much difficulty. The esophageal hiatus in the diaphragm is opened and the anterior vagal trunk is isolated and carefully dissected away from the lower esophagus. The anterior vagus is dissected distally to its initial branching to the anterior wall of the fundus of the stomach. The distal portion of the anterior vagal trunk traversing the lesser curvature is then divided. Further dissection of the anterior vagus and its proximal branches to the fundus may be necessary to provide an area for subsequent transection of the stomach. Some devascularization of the lesser curvature in this region may be necessary to accomplish this. The pouch will derive its blood supply from the uppermost branches of the splenic and gastroepiploic arteries which must be carefully preserved. Three or four atraumatic Babcock forceps are placed on the anterior wall of the fundus to tent it anteriorly. Two curved clamps are placed along the proposed line of division in such a manner as to include virtually the whole anterior wall of the fundus and very little posterior wall. Since no effort has been made to preserve the integrity of the fundic branches of the dorsal vagal trunk this maneuver is important because it results in a pouch consisting almost entirely of anterior fundic wall. A pouch with proportionately greater vagal innervation is thus ensured. The cannula is inserted in the usual manner and the pouch and stomach are individually closed in two layers, preventing internal fistulization.

C. *Rudick modification* (*19*): This pouch (Fig. 6) is essentially a modification of the vagally innervated total gastric pouch described by Dragstedt and Ellis (15), and consists of a vagally innervated pouch of the proximal stomach. Since mobilization of the vagal trunks and the lower esophagus entails opening of the pleural cavity, an endotracheal tube is passed and connected to a respirator at the outset of the operation. Construction of the pouch results in vagal denervation of the pyloric antrum. In order to prevent antral stasis, a pyloroplasty should be carried out; we prefer to do this as the first step of the operation. A long pyloroduodenal incision is made longitudinally (c) and closed transversely with a single layer of closely applied interrupted silk sutures.

The desired size of the pouch is determined. At the proposed line of transection (b), 1 to 2 cm areas are cleared on the greater and lesser

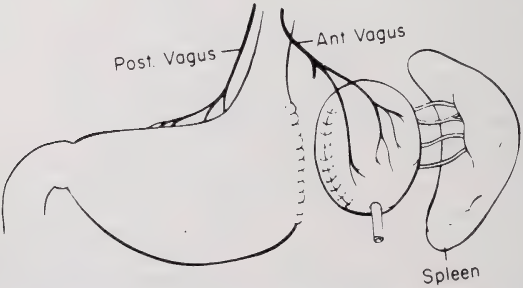

FIG. 5. Perry modification of Pavlov pouch. Pouch is innervated by anterior vagus and main stomach by posterior vagus.

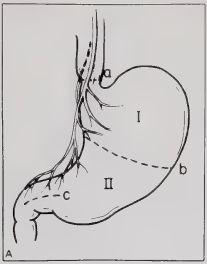

FIG. 6. Rudick modification of Pavlov pouch.
FIG. 6a. Shows lines of transection of esophagus (a) and corpus (b), with pyloroplasty (c).

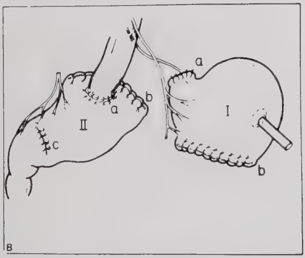

FIG. 6b. Shows completed pouch (I) and esophagogastric anastomosis into distal stomach (II), with pyloroplasty (c).

curvatures. The stomach is completely divided along this line; a Von Petz or similar stapling device facilitates this step. The distal (II) and proximal (I) parts of the stomach are completely closed. The distal part (II) consists of the entire antrum and a generous rim of corpus. Before closure of the proximal part of the stomach is completed, a gastric cannula is introduced into the pouch and brought out through its anterior wall in a dependent position.

Attention is now turned to the lower esophagus and vagus nerves. Traction is placed on the proximal stomach (1), and the phrenicoesophageal ligament is divided. Blunt finger dissection is used to free the lower esophagus and a soft tube is passed around it. The vagal trunks are identified and gently retracted away from the esophagus by umbilical tape: Two Satinsky clamps are placed on the lower esophagus (not the stomach) taking care to avoid including the nerve trunks in the clamps. The esophagus is transected at (A) and the distal (pouch) end completely closed; this completes the isolation of the pouch (I). Esophagogastric continuity is restored by anastomosing the upper divided end of the esophagus to the distal stomach (II) in an end-to-side fashion. The distal stomach tends to assume the shape of an elongated tube. The crura of the diaphragm are sutured to the stomach (carefully avoiding inclusion of the vagi in the sutures) to prevent herniation of small bowel into the chest. The chest is emptied of air, the pouch cannula is brought out through the abdominal wall in customary fashion to facilitate dependent drainage, and the abdominal incision is closed.

Like the pouch of Perry, this preparation of the oxyntic gland area is free from the problem which so frequently besets vagally innervated fundic pouches, namely, fistula formation between the pouch and main stomach. However, in the Perry modification only the anterior wall remains fully innervated, whereas the posterior wall is denervated. Although the degree of innervation can be increased by constructing the pouch so that it consists predominantly of anterior wall with less posterior wall, a certain degree of neuropraxia may still occur because of the tedious dissection attendant upon isolation of the vagi along the lesser curvature. In the present preparation, on the other hand, the vagi are easily identified and readily isolated with a minimum of trauma; total vagal innervation is thus possible. Apart from the Perry and Rudick techniques, there is an appreciable incidence of fistula formation between stomach and pouch. Care must be taken in the placement of the cannula when using the techniques with a mucosal septum, so that it does not encroach on the suture line of the mucosal septum, predisposing to fistulization. The loss of fluids and electrolytes is much greater than with denervated pouches, requiring greater care of the animals. Secretory responses may fluctuate more greatly than denervated pouches, as psychic influences may alter the secretory pattern.

Innervated antral pouches; These pouches are prepared by constructing a double mucosal septum between the antrum and corpus. The simplest method resembles the Thomas innervated fundic pouch. The antrum is completely divided from the duodenum just beyond the pyloric sphincter. Two Allis forceps are inserted into the antrum to grasp the mucosa at the incisura and the greater curvature at a point opposite the incisura. By gentle traction the antrum is everted and the mucosa presents as a rosette. The antrum corpus junction is delineated by the colorimetric method previously described. The mucosal septum is then constructed as for the Thomas pouch.

The following is an alternate method: The gastroepiploic vessels are divided at the junction of corpus and antrum. At this point a one centimeter incision is made through the seromuscular layer only on the anterior and posterior walls of the stomach. The mucosa is grasped with tissue forceps and undermined from the muscularis by scissors dissection. This is continued until the whole circumference of the corpus-antrum junction has been undermined. The mucosa at the junction is then incised and the anterior layer of the stomach side sutured to the posterior layer using a Connell suture. The anterior and posterior layers on the antral side are similarly sutured together, producing a double mucosal septum. The muscularis at the greater curvature is repaired with two or three sutures.

The antral pouch is usually matured as a mucocutaneous fistula as described earlier. It is customary to prepare a Heidenhain pouch simultaneously as an indicator pouch. Gastrointestinal continuity is restored by a gastroduodenal or gastrojejunal anastomosis.

Cervical Esophagostomy

Cervical esophagostomy may be used for purposes of feeding or for gastric intubation to study secretion or motility. When used for these purposes we find it best to introduce a cannula of the Thomas type (20). A midline vertical incision is made in the neck and the esophagus is mobilized extensively. An incision is made in the left anteriolateral aspect of the esophagus and the cannula is inserted; it is now sutured in place. The cannula is exteriorized through a separate stab wound and the main incision is closed.

Esophagostomy is also frequently required for the stimulation of the cephalic phase of gastric secretion by sham-feeding. It is imperative that all swallowed food and saliva pass out of the esophagostomy and not reach the stomach. Perhaps the best technique in our experience is that of Olbe (14). Parallel vertical incisions are made along each side of the neck to form a skin flap. The esophagus is mobilized through the left side of the neck from pharynx to mediastinum. The skin flap is elevated and then wrapped around the esophagus; it is sutured to itself behind the esophagus with absorbable sutures since these will be difficult to remove at a later date. Because of this skin flap a large cutaneous defect results, but the lateral skin flaps can be undermined and sutured to each other. The esophagus is thus exteriorized and covered with skin and looks like the handle of a suitcase. Three weeks later, at a second operation, the subcutaneous esophagus is opened and a cannula inserted. During experiments requiring sham-feeding the esophagus is occluded distal to the cannula with a soft bandage and the cannula is opened. All food and saliva will pass through the cannula and none will enter the stomach. The animals tolerate this preparation very well. When the cannula is closed, all swallowed food passes into the stomach.

The Cannula

The cannula utilized in these studies is constructed of stainless steel lined with nylon (Fig. 7) (3, 21). A suitable, alternate cannula is described by Gregory (22). A sharp-pointed nylon tip (c) is screwed onto the cannula (a) which is introduced into the pouch before the suture line has been completed. The sharp tip is forced through the wall of the pouch so that a snug fit around the cannula results; a separate incision is thus avoided. One or two pursestring sutures are placed around the cannula, around which omentum is then wrapped. A large nylon washer (d) is placed around the cannula outside the pouch and the sharp tip removed. A blunt nylon tip (e) is attached to the cannula; it has a threaded lumen into which a pull-through instrument (f) can be screwed. The pull-through instrument is forced into the peritoneal cavity from the external skin surface about 3–4 cm to the left of the midline. It is screwed into the blunt nylon tip and forcibly pulled out, pulling the cannula with it. This ensures a snug fit around the cannula. All external attachments

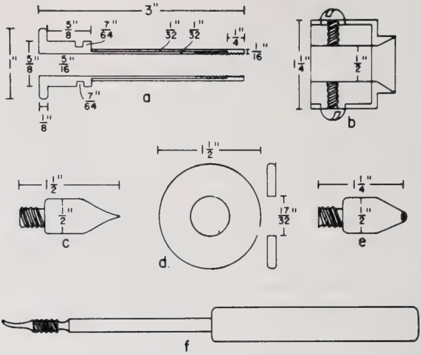

FIG. 7. Component parts of gastric cannula: (a) cannula; (b) bite block; (c) sharp-pointed nylon tip; (d) nylon washer; (e) blunt nylon tip with thread in lumen; (f) pull-through instrument.

of the cannula are removed and the bit block (b) attached. It is usually advantageous to place a second nylon around the cannula outside the skin prior to attaching the bite block.

The cannula must be constructed of an inert material and should not produce pressure necrosis of the pouch, which may result in leakage of secretions. The nylon washer, which distributes the weight of the cannula and collecting apparatus to a much larger area, is an additional factor preventing this complication.

Another eminently satisfactory cannula used by many investigators is the one devised by Gregory (6). It consists of a stainless steel or vitallium cylinder with a small notched inner flange; the notch facilitates insertion of the cannula through a stab wound in the wall of the pouch. There is a larger outer flange which is fenestrated to permit attachment to the serosa of the pouch by suture. Rotation of the cannula is thus prevented and adherence of the pouch to the anterior abdominal wall is enhanced. Traction on the cannula exerts traction on the abdominal wall and not the pouch wall.

Whatever type of cannula is used and whatever type of pouch is constructed, it must be emphasized that the cannula should not be brought out through the main incision. Problems with failure of healing of the incision and wound infection may occur. It is also desirable to get a snug fit around the cannula and this is impossible when it is brought out through the main incision. *We therefore advocate strongly that the cannula be brought out at a point separate from the abdominal incision.*

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The Use of Gastric Pouches in Gastric Physiology: II. Maintenance of Animals and Pouches*

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In the preceding paper (1), we described the anatomy of the stomach and the technical aspects of constructing gastric pouches. In this paper, we discuss the preoperative and postoperative management of these animals as well as their maintenance.

In general the dog is preferred because of its convenient weight, because its nutritional requirements are fairly well known and readily satisfied, and because it can be trained to follow simple routines. The dog can tolerate a gastric cannula for long periods while the large size of its stomach allows the construction of various types and sizes of pouches. Mongrels are generally preferred because they are hardier than pure breeds. Small dogs should be avoided because of the difficulty of constructing a large volume pouch. The standing height of the dogs must also be sufficient to permit the collecting apparatus for gastric juice to hang free without dragging. Short-haired animals permit easier preparation of skin and assure cleanliness during the postoperative collection period. If possible, the temperament of the animal should be assessed before constructing the pouch, since mean, irritable dogs may resist the frequent handling involved in collecting gastric secretions, and are more likely to chew on the collecting apparatus. Female animals are preferred because they are easier to keep clean and there is more room on the abdominal wall to bring out the gastric cannula. We have found females generally easier to handle during experiments. They also present less risk of contamination of dressings by urine. In males the penis and prepuce are prone to edema, especially in the presence of an abdominal cannula.

Preoperative Preparation

In our institution the gastric pouch dogs are housed in an environment in accordance with the principles outlined by the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." "Conditioned" animals are best suited for gastric pouch studies. The routine conditioning includes (a) general physical examination on arrival; (b) vaccination for distemper, canine hepatitis and leptospirosis; (c) dipping for ectoparasites; (d) fecal examination and deworming; and (e) heart worm screening. Good experimental data

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cannot be accrued if work is performed upon an animal that is suffering from disease or inanition. It must be emphasized that the nutrition of animals undergoing surgical procedures is as important as any other method in facilitating recovery. Animals should receive no food for at least 18–24 hours prior to operation, but they are never deprived of water. Complete emptying of the stomach in the dog usually takes about 8–10 hours, but lack of movement or exercise may prolong this physiological process. Gastric emptying in caged animals probably takes 12–18 hours. If, upon opening the abdomen, the stomach is found to contain food, the pouch should not be constructed because of the tendency to gastric retention and dilatation. Moreover, the dog tolerates spillage of gastric contents into the peritoneal cavity very poorly and rapidly develops peritonitis. Preoperative purgation is not advised; it probably does more harm than good by irritating gastric mucosa. It is suggested that all surgery ideally be performed early in the day as this insures adequate supervision of the animal during the phase of recovery from the anesthetic. Skin preparation is best carried out once the animal is already anesthetized. After shaving with fine electric clippers (Oster size 40 blade), the skin should be lathered well to remove dirt. Bactericidal soap is of advantage. This is followed by two applications of betadine or tincture of iodine or any suitable skin antiseptic. The abdomen is then draped in the usual manner.

The Incision

Adequate exposure is usually obtained with a midline linea alba incision extending from the xiphoid process to the umbilicus. This also lends itself to easy closure. When anticipating a difficult anastomosis at the lower end of the esophagus, or when the animal has a very narrow subcostal angle (males more frequently have narrow subcostal angles), it may be advantageous to utilize a long, left oblique incision. In such a situation, the animal should be placed with its hind limbs supine and its forelimbs in the right lateral position. The large ventral pad of fat which is found after incision of the posterior sheath is best excised as it may hamper future surgical procedures. Whatever incision is utilized, the upper limit should not extend above the tip of the xiphoid, as the slim anterior diaphragm may easily split with the retraction, or even with the incision, producing an open pneumothorax, an error that is easy to commit in the dog.

After completion of the procedure, the abdomen is closed in layers in the routine manner. Postoperatively the animal may lie in its own urine; nonabsorbable sutures for skin are probably advisable to reduce skin wound infection.

Postoperative Care

The animals should be turned every two hours after surgery to prevent the development of hypostatic pneumonia. Postoperatively, blood transfusions are not needed if meticulous hemostasis has been carried out. Dosages of 500–1000 ml intravenous 5% dextrose in 0.2% saline is administered daily for five days. This can also be administered subcutaneously because of the looseness of the skin of the dog, provided the total amount is not given into one area. Nothing is allowed by mouth for four days, during which time all fluids and antibiotics are administered intravenously, subcutaneously, or intramuscularly. On and after the fifth postoperative day, water is given freely. On the 6–7th day, very palatable food (e.g., baby food) as well as water is given. On the 8th day, four feedings are given consisting of 50 gm ground meat of a pureed consistency. The intake of meat is then increased to toleration, always maintaining a soft consistency until the animal is able to revert to his previous regular diet. Frequent small feedings are necessary since these animals have a reduced capacity of the stomach and may take some time to adapt.

After the first few weeks the amount of care necessary depends upon the amount of gastric juice draining from the pouch. Continuous and unreplaced loss of gastric juice may lead to dehydration and alkalosis. More severe effects are seen with larger (e.g., total gastric and vagally innervated) pouches, which secrete large volumes of gastric juice daily. At the one end of the scale, the Heidenhain pouch and gastric fistula preparations require very little care—a teaspoon of sodium chloride and a vitamin preparation daily. At the other end of the scale, total gastric pouch (innervated) preparations can only be maintained for long periods with meticulous care; in addition to the dietary care, 500–1000 ml of normal saline should be administered intravenously or subcutaneously. The daily quantity of gastric juice, either in its native form or as 0.1 N HCl, should be returned each day with the food. Some preparations may even require an esophagostomy so that the animals may be fed by tube. A sample diet for vagally innervated pouches is given in Table I (2). It is unnecessary to add vitamin C, as dogs manufacture endogenous ascorbic acid. The type of diet may affect the secretory activity of the pouch; high protein diets cause a greater daily output of acid (3).

It is of the utmost importance that the animals maintain a constant body weight, as fluctuations in weight may alter the secretory responses. All animals should, therefore, be weighed weekly. Unexplained weight loss is frequently the first sign of ulcer formation (especially in the ulcerogenic preparations) or of small bowel obstruction.

Each animal is kept in a separate cage with mesh bottoms to allow the passage of excreta, since some animals may be coprophagic. It is important, howver, that they be exercised at least once daily.

Secretory studies should not be commenced for at least three or four weeks after surgery, as it takes this amount of time for the pouches to become standardized.

With any pouch the cannula should be kept open at all times unless a collecting apparatus is attached to the cannula; this will prevent distension of the pouch. With fistulae, on the other hand, the cannula must be kept permanently closed (except during experiments) or the animal may become rapidly dehydrated and emaciated.

TABLE I

Pavlov Pouch Diet

A. *Basic Diet.* 20 gm per kilogram body weight per day (adjusted as necessary to maintain constant weight)

1. Mix together the dry ingredients:

Sugar	4,500 gm
Salt	300 gm
Salt mix No. 2*	300 gm
Casein	2,500 gm

2. Add these oils to the dry ingredients and mix thoroughly:

Cottonseed oil	1,200 gm
Vitamin K (see below)	60 gm
Vitamins A & D (see below)	30 gm
Cod liver oil	144 gm

3. Add water—3,000 gm and mix for 15 to 30 minutes.

Vitamins A & D†	2 gm vitamin A 0.5 gm vitamin D	+ 226 ml cottonseed oil
Vitamin K†	1 gm + 1,000 ml cottonseed oil	

B. *Vitamin Supplement.* Each dog receives 10 ml per. day.

Nicotinic acid	8 gm	Riboflavin	400 mg
Calcium pantothenate	2.4 gm	Pyridoxine HCl	200 mg
p-Amino benzoic acid	2.0 gm	Folic acid	100 mg
Choline chloride	40.0 gm	Vitamin B_{12}	4 mg
Inositol	40.0 gm	Biotin	200 mg
Thiamine HCl	200 mg		

To total weight of dry ingredients (93 gm), add 3,907 ml water and store in refrigerator.

The daily amount of pouch diet plus 10 ml of vitamin supplement is placed in plastic storage cups, which are covered tightly and refrigerated. Cups are labeled for each dog. A three-week supply is prepared at one time.

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* Nutritional Biochemical Corporation.

† These should be stored in brown bottles and kept refrigerated.

Common Complications and Their Management

1. The early complications are similar to those attendant upon any abdominal operation, in particular, hypostatic pneumonia. Frequent turning of the animal postoperatively is essential. The addition of postoperative antibiotics is a useful adjunct.

2. Dehydration from loss of fluids and electrolytes is a complication that should be avoided. The gastric fistula and esophageal fistula animals should have the cannulas capped at all times (except during experiments). Unless the cannulas are examined daily, the absence of a cap may easily go unnoticed. Prolonged use of some cannulas may result in a loosely fitting cap; this should therefore be checked carefully before using it in any animal. Some animals also chew on the cannulas, displacing the cap. Clearly the best treatment is prevention. Vigilance will be more rewarding than replacing the fluid losses which may be severe enough to produce rapid inanition and death.

Excess loss of fluids also occurs in the vagally innervated fundic (Pavlov) or total gastric pouches. Large volumes of highly acidic gastric secretion are secreted daily. This makes management of animals with these pouches more difficult to manage than the denervated pouches or the fistula animals. Parenteral fluids should be given as a supplement or the gastric juice secreted on the previous day should be added to the food.

3. Distension of the pouch may occur if the cannula is capped in a closed system. The cannulas of the pouches should, therefore, be kept open continuously unless a collecting apparatus is attached to the cannula. Distension of the pouch results in ballooning of the pouch with atrophy of the secreting cells and increased mucosal permeability to acid.

4. Acid-peptic ulceration of the skin very seldom occurs on the abdomen, but may occur on the legs when acid and pepsin pour over it continuously. The animals should be trained to keep the cannula (and pouch secretions) away from the legs. In the interim a collecting device (a football bladder is ideal) is connected to the cannula and all gastric juice is diverted away from the skin. Frequent bathing of the sores is carried out and topical antibiotic creams are applied. A paste made of Furacin® (Eaton Laboratories) and Morusan® (Massengill) in equal parts is very useful when applied to the affected area. It is very rare that the sores will not heal with this regime.

5. Whenever using preparations, e.g., gastric fistulas, in which the cannula must remain capped, particular attention must be paid to the patency of the cannula. Collection of food debris within the lumen of the cannula may result in caking in the cannula. The complete collection of gastric secretion may thus be impaired. In the fistula preparations it is advisable to flush the cannulas with water or saline at least twice a week.

6. Extrusion of the cannula results if adequate care is not taken in the placement of the cannula. A snug fit in the pouch and through the skin is essential. The cannula should never be exteriorized through the main incision. Two washers, one between pouch and peritoneum and one between skin and bite block are essential. Even though the animals may chew incessantly on the cannulas, the cannula will remain intact if the above principles are adhered to. On a rare occasion the bite block has become caught in the mesh of the cage floor. Even violent tugging by the animal has not usually resulted in the cannula being pulled out. Unless the internal nylon washer is used, this can easily occur.

When the cannula is extruded or pulled out then the abdomen is reexplored. By this time the pouch has usually become well sealed off from the rest of the abdomen so that peritonitis is not a usual result of this complication. The original cannula opening is completely closed with two layers of sutures and the cannula is replaced *at a different site* on the anterior wall of the pouch.

Our experience shows that extrusion of the cannula occurs regularly if the cannula is brought out through the wall of the antrum or is placed in an antral pouch. The intense muscular contractions of the antrum prevent a snug fit from remaining so for long in the antrum. All the cannulas inevitably are extruded from the antrum although the cannula may still remain deep to the abdominal wall. This leads to a false sense of security but the pouch is of no value for further experiments. It can be easily recognized by instilling a given volume of saline through the cannula into the pouch. Unless the identical amount (or more) fluid is recovered this complication should be suspected. The pouch is then examined under fluoroscopy while instilling radiopaque dye through the cannula. Because of the small size of the antral pouches it is very difficult to repair them and we generally discard these pouches when complications occur.

7. Fistulization from the pouch to the main stomach is a frequent complication of Pavlov pouches. Any pouch using a mucosal septum is beset with this problem, which occurs regardless of how meticulous one is in the construction of the pouch. Since there will be admixture of the contents of the main stomach and the pouch, the experimental results will be invalidated. Repeated x-ray of the pouch filled with radiopaque material, barium or gastrografin, is used to identify this complication. There is no satisfactory way to correct this and the animal will have to be excluded from further study. The techniques of Perry (4) and Rudick (5) avoid fistulization in vagally innervated pouches.

8. Occasionally the cannula may cause erosion of the pouch with infection of the mucosa. Pouch secretions may then drain around as well as through the cannula, invalidating all results. The presence of infected material in the secretion also alters the titratable acid. With mild infections, irrigation of the pouch with antibiotics may suffice, but reoperation with repair of the pouch and reimplantation of the cannula may be necessary. It must be remembered, however, that these pouches will have to be restandardized with repeated control studies.

9. Weight loss should not be a problem with fistula animals and with vagally denervated pouches. These animals generally maintain their weights very well with a minimum of care after adapting to their "new physiology." Loss of weight results from loss of appetite, vomiting or some other condition. In our experience, unexplained weight loss is a frequent heralding sign of peptic ulceration in the stomach, duodenum or jejunum, of intestinal obstruction or of early peritonitis. When an animal loses weight inexplicably it is rash to say "increase the food intake" until these possibilities have been completely excluded. Moreover, increasing the food intake may actually aggravate the weight loss by causing vomiting.

10. Vagally denervated pouches are subjected to less instantaneous variation in pouch output than the innervated preparations. When the latter are studied they should therefore be freed from all extraneous distractions during the experimental procedure. Even the sight or smell of food will influence the results.

11. Occasionally, irritable dogs bite excessively at the cannula or at the collecting device. This can be managed in two ways: A canvas sling is kept around the cannula, but this may inhibit experiments. A restraining (Elizabethan) collar can be placed around the neck. We have found the use of a small inflatable rubber inner tube to be most effective. When the tube is inflated

after being placed around the dog's neck, it prevents the animal from bending its neck and reaching the abdomen with its teeth.

Finally, patience and understanding of animal behavior, using "moral restraint" when necessary, will pay dividends in terms of the investigator's time, money, and effort in this phase of animal experimentation.

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Surgical versus Injection Treatment for Varicose Veins

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Varicose veins are one of mankind's commonest afflictions. Almost every medical practitioner is confronted at some time by varices or their complications. Man has not yet adjusted to the upright position and he pays a penalty because of the strain on his back and lower extremities. The veins of the lower extremities are rarely diseased in youth. It is usually in the middle and later decades that the abnormalities due to chronic venous congestion appear. Although the exact etiology of varicose veins remains obscure, a familial predisposition is probably the most important cause. Obesity, pregnancy, and prolonged dependency of the legs are all aggravating factors.

The fact that diverse suggestions for treatment of varicose veins are still being proposed is prime evidence that the ultimate in therapy has not yet been achieved. It is true that the exact etiology of varicose veins remains obscure and that the disease tends to be progressive. Nevertheless, certain basic abnormalities prevail in the vast majority of patients and if appropriate therapy is instituted, excellent results can be anticipated.

Injection treatment has been used for varicose veins for many years. Linser, Sicard, Sigg, Tournay and many others advocated injection therapy for the treatment of varicose veins in the early part of this century. The development of Sotradecol® (Product of Elkins-Sinn, Inc., Cherry Hill, New Jersey) as a sclerosing agent, has greatly improved the results of injection therapy. It is a sodium salt of tetradecyl sulfate. Pierpont and Brantigan (1) and Cooper (2) found it less toxic and more effective than other sclerosing agents available. This potent thrombogenic action and the relative absence of side-effects have been confirmed by Dingwall, Lin, and Lyon (3). The solution is clear, nonviscid, has a low surface tension, and is readily miscible with blood, thus providing a uniform distribution following injection. A three percent solution is used for large varices, while the one percent concentration is used for "spiders" and small varicose veins. The technique of injection suggested by Orbach (4) has proven effective. Dilution of the sclerosing solution by blood is minimized by introducing a small air bubble or foam into the lumen of the vein immediately preceding the injection. There is no danger of air embolism and the results reveal a high incidence of obliteration. Minimal thrombosis should be achieved and methods should be used which produce venous obliteration by intima concretion and not by organization of large thrombi. In 1963 Fegan (5) described a modified injection technique which involves continuous compression of the injected varicose veins. Stuebner (6) described

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the use of effective injection technique, coupled with conservative surgical procedures. Chant, Jones and Weddell (7) carried out a study to determine the comparative results following the treatment of varicose veins by surgery versus injection. "The results at three years after treatment showed no difference between the two methods of treatment." Piachaud and Weddell (8) did a study to estimate the comparative cost to the British National Health Service, the patient, and the community when treatment was carried out by injection versus surgery. It was concluded that it would be more economical to use injection treatment for varicose veins. According to Hobbs (9), "it is clear that there is a place for both surgery and injection in the treatment of varicose veins and the physician must be competent in both methods and equally enthusiastic if he is to select the most effective plan for treatment." After developing a reliable injection technique, this author compared it with standard surgical procedures in a random trial. "The results showed that after one year 82 percent of unselected patients were cured by injections but after six years the cure rate was only seven percent. The surgical result was not as good at one year but much better than injection after six years." "The best primary treatment for dilated superficial veins and for incompetent perforating veins in the lower part of the legs was injection-compression. However, surgery was much more successful and long-lasting when there was involvement of the saphenous systems with proximal incompetence.

Some of the conclusions arrived at by authors advocating the sole use of sclerotherapy are based on the assumption that a patient who has surgery for varicose veins must be hospitalized for three to seven days and is then unable to work for several more weeks or longer. At present, a complete stripping for varicose veins can be performed on a fully ambulatory basis (10). The procedure can be performed under local or general anesthesia. The patient is admitted at 7:30 A.M. and is discharged at 3 P.M. that same day. He is instructed to return to work and all usual activities the day after surgery. With this technique there has been a great saving of hospital beds and work-hours lost to industry. Each patient is different and should be treated individually. If there are large varicose veins and marked valvular insufficiency at the sapheno-femoral or short sapheno-popliteal vein junction (Figs. 1, 2), operative treatment has been very effective. On the other hand, if the varices are scattered and there is no retrograde flow resulting from severe valvular insufficiency (Fig. 3) injection treatment is very effective. Very large varices due to marked valvular insufficiency, especially along the saphenous system, tend to recanalize after treatment by injections. Of course there are some patients in whom the indication for surgery versus injection is not crystal clear. Most patients fall into either the surgical or injection group and this differentiation can usually be made by careful inspection and palpation, with the patient standing. The operative results are not satisfactory unless each and every site of valvular insufficiency is ligated flush with the deep veins. In order to compare surgery and injection treatment, the surgical technique considered must be meticulous. The success of operation depends proportionately upon the ability of the surgeon to detect and excise all sites of valvular

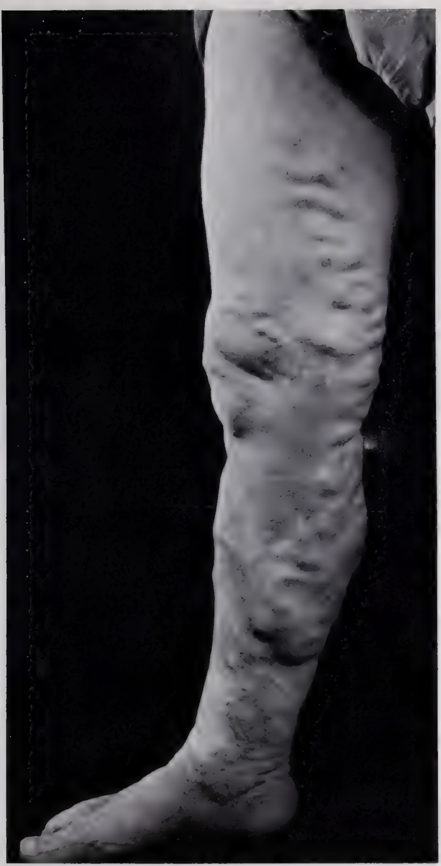

FIG. 1. Marked insufficiency along long saphenous vein. Since predominant retrograde flow originates at sapheno-femoral junction, best results are obtained by high ligation flush with femoral vein, followed by stripping of long saphenous vein.

insufficiency at their source. Many patients have been operated upon several times and large varices continue to recur because the saphenous vein has not been ligated flush with the femoral or popliteal vein or incompetent perforators have been missed (11).

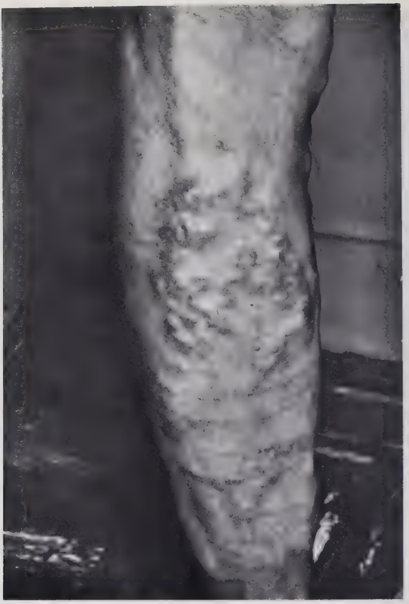

FIG. 2. Marked valvular insufficiency at short sapheno-popliteal vein junction, resulting in dilated short saphenous vein. Excellent results can be achieved by ligation of short saphenous vein flush with popliteal vein, followed by stripping procedure. Entrance into popliteal vein is usually about 1/2 inch above popliteal crease. In this case it is much higher than usual.

The ideal operative procedure for varicose veins includes the following features (12): 1) elimination of all venous incompetency at its source; 2) ligation of the larger communicating veins so that there is minimal postoperative bleeding; 3) extirpation of all residual varicose vein segments so that few, if any, postoperative injections are required; 4) making as few incisions as possible so that the operating time is short and the cosmetic result good; 5) mobilization of patient shortly following surgery to minimize postoperative complications. A surgical technique has been devised which meets these criteria. In the majority of patients four incisions are all that is required. The incisions in the thigh and leg can be made vertically and very short (½ inch) because the exact course of the veins has been previously delineated by the skin marking. Only in the groin and knee region are transverse incisions made. The scars that remain are practically invisible. If incompetent perforators are present, each must be dissected out and ligated flush with the deep veins

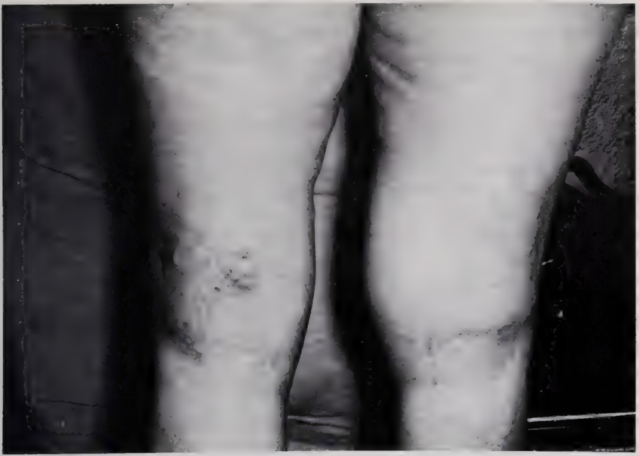

FIG. 3. Varicose veins not associated with marked sapheno-femoral or sapheno-popliteal vein insufficiency or markedly incompetent perforator. Surgery is not necessary. Excellent results can be achieved with injection therapy.

before the stripping is carried out. If incompetency exists in the short saphenous system, incisions are made vertically in the popliteal area (from the popliteal crease upward) and calf in similar fashion to eradicate all venous insufficiency.

With the ambulatory surgical technique there is little disruption of family life and an elastic support is only necessary for seven to twelve days postoperatively. With the injection/compression technique, the bandage is used for about six weeks. This extended use of the bandage would not be acceptable to many patients. In view of the fact that the entire operative procedure can be performed on a fully ambulatory basis and that only about two postoperative visits are necessary for suture removal and possible injections (for any small residual varices), the surgical procedure is actually less disruptive of a patient's usual activities than injection therapy. It is certainly preferable, if there is a choice, to remove the diseased vein segments, rather than inject them and leave the venous cords in the leg. The ambulatory procedure also greatly reduces postoperative morbidity and mortality, especially the incidence of thrombophlebitis.

The advocates of sclerotherapy state that the patient is "saved" from an operation. Actually, many patients who have had both operative and injection treatment have stated that the injections caused more pain and disability than the operation. Minimal postoperative reaction develops if the patient is fully ambulatory shortly after operation. Not only are all incompetent veins eliminated at operation, but by stripping out the saphenous vein, other communicating branches are torn, thus decreasing the possibility of incompetency developing at these sites at a future date.

The problem often arises as to whether or not treatment should be carried out for varicose veins in the presence of deep venous disease. Varices alone do not produce significant edema. The presence of edema, not systemic in origin, usually indicates deep vein insufficiency. Some believe that the varices in these patients act as collateral vessels and that their excision would aggravate venous insufficiency. Experience has not borne this out. In any patient in whom retrograde blood flow can be demonstrated, regardless of the status of the deep circulation, the usual benefit can be anticipated after the stripping of large varicose veins. According to Homans (13), varicose veins do not forward venous blood against gravity. A collateral circulation by way of nonvaricose veins must be established after deep phlebitis if the leg is not to remain congested and cyanotic. Obliteration of varicose veins is therefore beneficial because the varices are actually contributing to venous stasis in the leg. If the predominant pathology in the leg is marked edema and the varices are secondary in nature or moderate in size, then surgery would not be indicated because it would not help the edema, which is due to deep vein disease.

The results of operation have been gratifying. No significant postoperative bleeding has occurred, even though patients have been fully active several hours after operation. There has been occasional induration of the operative wounds but no infection or hematoma sufficiently severe to require drainage. Apparently, active walking shortly after operation and full activity the following morning are major factors in preventing postoperative complications. Annual follow-up studies have revealed that about half the patients do not require any injections after the immediate postoperative period. In the other half, small varices develop, especially if the patients have spider veins, which tend to become larger with the passage of time. These varices can be easily eliminated by injections of sclerosing solution.

Conclusions

Since surgery can be performed on an ambulatory basis, it is important to disregard economic and other nonmedical factors and concentrate on specific indications for treatment of varicose veins. All patients cannot be placed in the same mold and treated in the same way. It is true that, with increasing experience, a physician becomes more proficient and gets better results, whether injection or surgical treatment is used. Long-term studies are essential to determine the results of treatment for varicose veins since varices tend to recur. With marked valvular insufficiency along the saphenous veins or from markedly incompetent perforators, ligation and stripping is more effective since the proper ligation of incompetent sites immediately eliminates retrograde venous flow. Injection treatment is preferable when retrograde venous pressure is not very marked. These varices are usually superficial but they can become quite large. In the ideal case for injections there is no marked venous insufficiency within the long or short saphenous vein. With both types of therapy the patient should be ambulatory since this is the best way to prevent complications. If each patient is individualized and treated according to specific indications, excellent results will be obtained both from injection therapy and surgery. If proper primary treatment is performed and the patient is seen once a year, any small varices which develop can be eliminated by injections and the leg will remain free of varicose veins indefinitely.

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Modified Radical Mastectomy

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Introduction

Modifications of radical mastectomy that preserve one or both pectoral muscles are being employed more frequently for the treatment of early breast cancer. A recent report that compared results following modified radical mastectomy with those following conventional radical mastectomy for patients with Stage I and Stage II breast cancer demonstrated that these operations were followed by almost identical 5-year recurrence-free survival (3). Local recurrence was also similar following both operations. The modified radical mastectomy that was employed was that described originally by Patey (4). This procedure permits axillary dissection as complete as that accomplished by radical mastectomy. Other techniques of modified radical mastectomy which preserve the pectoralis minor as well as the pectoralis major do not achieve as thorough an axillary clearance. The proponents of these procedures maintain that more thorough axillary dissection is not really necessary but they have not as yet shown equivalent comparative survival statistics (2).

Since there are significant variations in technique, it was thought advisable to describe and illustrate the technique of the Patey operation in some detail indicating the factors which permit thorough clearance of all regional nodes. Preservation of the pectoralis major muscle does not interfere with thorough axillary dissection. It can be readily retracted to provide access to the apex of the axilla. Flexion of the shoulder joint facilitates this exposure. The barriers to complete axillary dissection are primarily the nerve supply to the pectoralis minor muscle which hampers access to the nodes immediately beneath the insertion of the pectoralis minor and the bulk of the pectoralis minor itself, which interferes with exposure of the apex of the axilla lying medial to the pectoralis minor muscle. The other potential inadequacy of the operative procedure is the presence of interpectoral nodes (Rotter's nodes) lying in the fat pad between the pectoralis major and the pectoralis minor. These nodes are quite small but can be involved relatively early in patients with tumors in the upper hemisphere of the breast. On several occasions when the interpectoral fat pad and nodes have been excised as a separate specimen, nodes in this area have shown metastatic deposits and on two occasions in a series of about 150 cases they were the only nodes to be involved.

Invasion of the pectoralis major muscle itself is a late complication of carcinoma. The pectoral fascia overlying the muscle acts as a barrier to spread

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of tumor. Tumors that are adherent to the pectoralis muscle are not suitable for this operation. If the tumor is on the deep surface of the breast, it is often wise to remove some of the substance of the pectoralis major immediately beneath the tumor in order to insure complete removal of the pectoral fascia.

Technique

The operation employed is illustrated in the accompanying figures. The incision, flap development and closure of the wound after modified radical mastectomy are identical to that practiced for standard radical mastectomy. Figure 1 illustrates one type of incision often employed. It circumscribes the breast with no or little extension either upwards toward the clavicle or laterally. Closure requires excision of triangular shaped segments of skin to fashion flaps and remove redundancies. The dissection is started on the lateral flap and continued to the lateral edge of the latissimus dorsi muscle which is readily identified. The skin flap is taken thin, the dissection is carried out in the plane external to the membranous layer of the superficial fascia, between it and the skin. The anterior edge of the latissimus is followed to the axillary vein where the latter enters the arm to become the subclavian vein. The medial and superior flaps are then dissected as in the classical radical mastectomy in the plane between the membranous layer of the superficial fascia and the skin. Superiorly the dissection goes to the clavicle and medially to the midline. At the superior and medial limits of the dissection the incision is carried through the pectoral fascia and is then continued laterally and inferiorly between the pectoral fascia and the pectoralis major muscle (Fig. 2).

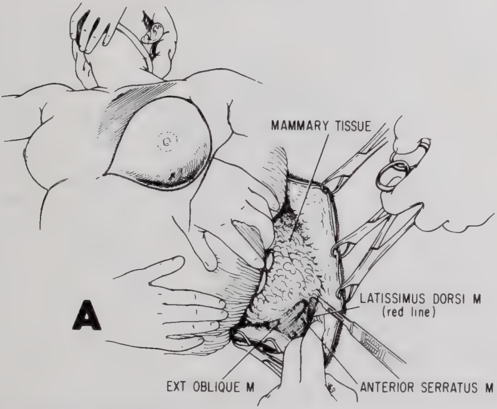

Fig. 1. Incision and development of skin flaps. Skin flaps are thin and taken laterally to the anterior edge of the latissimus dorsi, superiorly to the clavicle and medially to the midline.

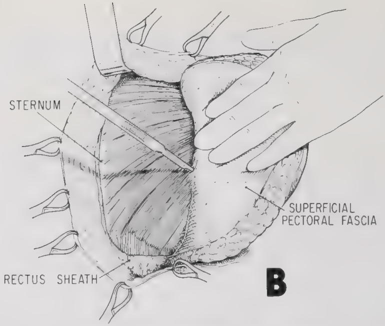

FIG. 2. Dissection of the breast from the pectoralis major muscle. The dissection is performed between the pectoral fascia and the pectoralis major muscle.

During this dissection the anterior terminal branches of the posterior intercostal vessels and the perforating branches of the internal thoracic vessels are secured as they emerge from the pectoralis major muscle and are ligated.

The dissection is continued to the free edge of the pectoralis major at which point terminal branches of the pectoral branch of the thoraco-acromial vessels are ligated. The dissection is continued on the deep surface of the pectoralis major muscle. The terminal branches of the lateral thoracic vessels as they pierce the pectoralis minor to enter the deep surface of the pectoralis major are divided. With these vessels the terminal branches of the medial pectoral nerve are encountered and these are similarly ligated and divided. This denervates a small part of the lateral fibers of the pectoralis major. Occasionally these fine filaments can be freed of surrounding fat, vessels and lymphatics, and preserved. The lateral pectoral nerve and the accompanying pectoral branches of the thoraco-acromial are identified on the undersurface of the pectoralis major and are preserved. The dissection must be meticulous and close to these structures in order to assure removal of all the interpectoral nodes. Dissection is continued until these structures are encountered where they emerge through the clavipectoral fascia medial to the insertion of the pectoralis minor. Laterally the axillary fascia is divided from the lateral edge of the insertion of the pectoralis minor to the axillary skin. In order to facilitate this exposure, the shoulder and elbow are flexed, the arm is elevated and placed across the patient's face as illustrated in Figure 3. This maneuver relaxes the pectoralis major muscle and permits its complete retraction medially.

Figure 4 illustrates the division of the insertion of the pectoralis minor which completes the exposure of the axillary contents. Simple division of the pectoralis minor is sufficient to provide exposure of the axillary contents; however, if the proximal stump is left attached to its origins a prominent nodule can subsequently be felt in the axilla. This nodule may be confused

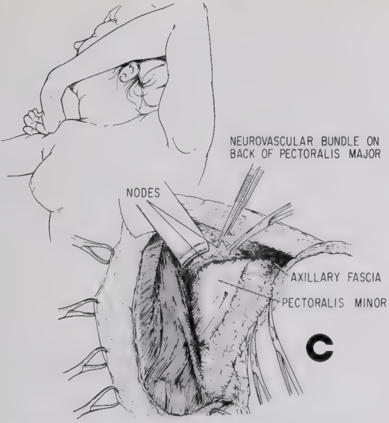
NEUROVASCULAR BUNDLE ON BACK OF PECTORALIS MAJOR
NODES
AXILLARY FASCIA
PECTORALIS MINOR
C

FIG. 3. Dissection of the interpectoral space. The dissection is continued on the deep surface of the pectoralis major, carefully removing all fat, areolar tissue, lymphatics and lymph nodes from the undersurface of the pectoralis major. The pectoral branch of the thoraco-acromial vessels and the lateral pectoral nerve are preserved and cleared of surrounding fat and lymphatics. Insert: Position of arm during the dissection.

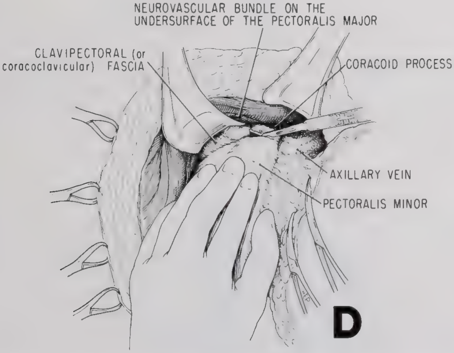
NEUROVASCULAR BUNDLE ON THE UNDERSURFACE OF THE PECTORALIS MAJOR
CLAVIPECTORAL (or coracoclavicular) FASCIA
CORACOID PROCESS
AXILLARY VEIN
PECTORALIS MINOR
D

FIG. 4. Division of insertion of pectoralis minor and exposure of the axillary contents.

with recurrent tumor in axillary nodes. Axillary dissection then proceeds from medial to lateral. The tributaries of the axillary vein are secured close to the axillary vein, the pectoral branches of the thoraco-acromial vessels and the lateral anterior thoracic nerves are spared but the adjacent lymphatic, areolar and fatty structures are carefully dissected from the undersurface of the pectoralis major muscle (Fig. 5). The nodes at the apex of the axilla are dissected free and the dissection is continued laterally. The long thoracic and thoracodorsal nerves are identified and preserved. The other axillary structures are removed. The dissection is completed by dividing the lateral perforating branches of the intercostal vessels and nerves on the serratus fascia. The status after completion of the dissection is shown in Figure 6. Skin closure will vary with the skin incision that was made. If the ovoid incision illustrated in Figure 1 is used, redundant triangular segments of skin at the angles are removed and the incision is closed in a Y fashion. Suction drains are

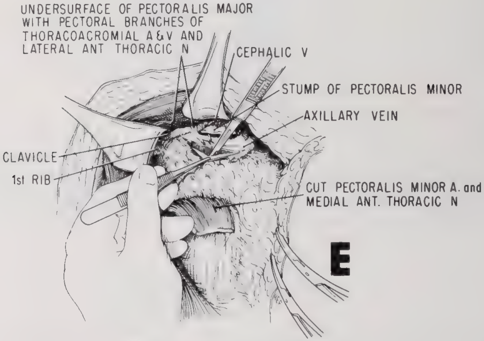
UNDERSURFACE OF PECTORALIS MAJOR WITH PECTORAL BRANCHES OF THORACOACROMIAL A & V AND LATERAL ANT THORACIC N
CEPHALIC V
STUMP OF PECTORALIS MINOR
AXILLARY VEIN
CLAVICLE
1st RIB
CUT PECTORALIS MINOR A. and MEDIAL ANT. THORACIC N
E

FIG. 5. Axillary dissection. Division of the branches and tributaries of the axillary vessels.

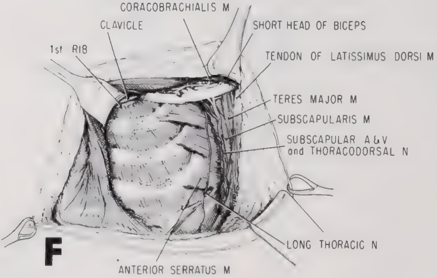
CORACOBRACHIALIS M
CLAVICLE
SHORT HEAD OF BICEPS
1st RIB
TENDON OF LATISSIMUS DORSI M
TERES MAJOR M
SUBSCAPULARIS M
SUBSCAPULAR A & V and THORACODORSAL N
LONG THORACIC N
F
ANTERIOR SERRATUS M

FIG. 6. Status after axillary dissection: The stumps of the origins of the pectoralis minor, the stumps of the perforating branches of the intercostal vessels and intercostal nerves, the long thoracic and thoraco-dorsal nerves cleared of surrounding structures.

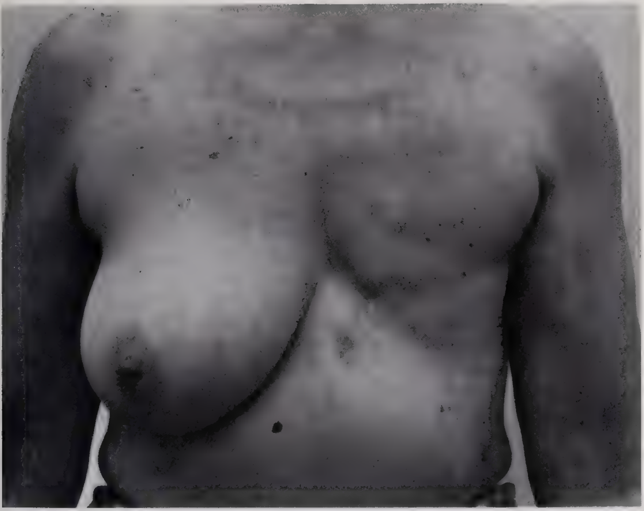

FIG. 7. Postoperative status after healing of the wound.

placed into the medial and lateral portions of the incision and are attached to some form of suction drainage.

When first trying this technique, one is usually surprised at how complete the dissection can be. Division of the lateral thoracic vessels and the medial anterior thoracic nerve along with division of the pectoralis minor open up the axilla to a thorough dissection. Appearance after healing is shown in Figure 7.

Discussion

Mastectomy with axillary dissection without removal of the pectoral muscles was first introduced by Charles H. Moore, Surgeon to the Middlesex Hospital, in 1867. Patey and Dyson (4) reintroduced the operation in 1948. They described all the features necessary for complete axillary dissection. They presented comparative 3-year survival rates and noted that there was no significant difference. However, one would not expect a difference in survival secondary to the operative procedure to be noticeable in this length of time. Any difference would rather be attributable to differences in the extent of disease in the patients treated by the different methods.

Handley and Thackray (1) in 1969 published 5- and 10-year results following modified radical mastectomy performed for 143 patients. They noted 75% 5-year survival in patients with stage A disease of the Columbia Clinical

Classification and 57% 5-year survival for the patients with Stage B disease. The 10-year rates were 61% for Stage A and 25% for Stage B. The latter rates were compared with the results of conventional radical mastectomy performed at several other centers. The results were similar. Papatestas and Lesnick (3) in 1974 compared 5-year survival rates following modified radical mastectomy with the survival following conventional radical mastectomy in patients with Stage I and Stage II tumors of the International TNM Clinical Staging System and found virtually identical survival rates of 84% following modified radical mastectomy and 81% following conventional radical mastectomy.

All of the above authors commented on the low incidence of local and regional recurrence which did not differ from that noted following conventional radical mastectomy, as well as on the absence of recurrence in the pectoralis major muscle. They all concluded that, because of improved appearance and function with preservation of the pectoralis muscle, the operation is preferred for operable breast cancer that does not involve the pectoral muscles and does not display the features of locally advanced disease that would entail classification as Stage III of the International TNM System or Stage C or D of the Columbia Clinical Classification.

Summary

A technique of mastectomy with preservation of the pectoralis major muscle and division of the pectoralis minor that permits thorough axillary dissection is described. This operative procedure has yielded recurrence-free survival rates identical to those following conventional radical mastectomy in a comparative study of these two procedures.

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Carcinoma of the Small Intestine in Crohn's Disease—Occurrence in a Bypassed Loop

UNSUP KIM, M.D., MARK KLEIN, M.D., SE-MIN BAEK, M.D., ALEXANDER RICHMAN, M.D., AND ARTHUR H. AUFSES, JR., M.D.

Abstract

A case of adenocarcinoma which developed in the proximal small intestine, previously bypassed for Crohn's disease, is reported. The authors feel that chronic severe cicatrizing inflammation and stasis of bowel contents in the blind loop, in this particular patient, may have contributed to the development of carcinoma.

The question of whether chronic inflammatory disease of the bowel (Crohn's disease) predisposes to the development of carcinoma remains unsettled (1-4). Chronic cicatrizing inflammation of the bowel, changes in composition of bowel contents, depressed immunologic response, and slow transit or stagnation of potentially carcinogenic substances in excluded or bypassed loops of diseased bowel may be contributory factors.

The following case report deals with a patient with Crohn's disease of small intestine, who developed adenocarcinoma in a previously bypassed loop of severely diseased proximal small bowel. Could the factors mentioned above have played a role in the development of the adenocarcinoma?

Case Report

J. M. (MSH #084442), a 52-year-old Jewish white man, was admitted to The Mount Sinai Hospital February 5, 1975, with a two-day history of abdominal pain. The history of gastrointestinal problems dates back to childhood, when the patient started having intermittent episodes of abdominal cramps not associated with diarrhea. Complete investigations at various hospitals were allegedly negative and he was diagnosed as having a "nervous stomach." In January 1971 laparotomy was performed at The Mount Sinai Hospital for partial small bowel obstruction. The small intestine was diffusely involved from proximal jejunum to proximal ileum, with multiple areas of stenosis beginning at 4.5 feet from the ligament of Treitz and extending five feet. The distalmost four feet of ileum appeared normal. Because of the length of the involved segments, a side-to-side jejunoileostomy was performed to relieve the obstruction, rather than a resection of a long segment of bowel. The patient did well postoperatively and was symptom-free for one year following surgery, at which time his abdominal cramps returned. He was treated with azulfidine, prednisone and bland diet. Two days prior to the present admission, the patient developed the acute onset of severe abdominal pain associated with vomiting and fever.

Physical examination on admission revealed an apprehensive male in acute distress. The temperature was 100.2°F, the blood pressure was 100/60 mm Hg, and the pulse was 88 per minute. Pertinent physical findings were confined to the abdomen. There was moderate tenderness in the left lower quadrant without spasm or rebound tenderness. Abdominal flatplate showed distended

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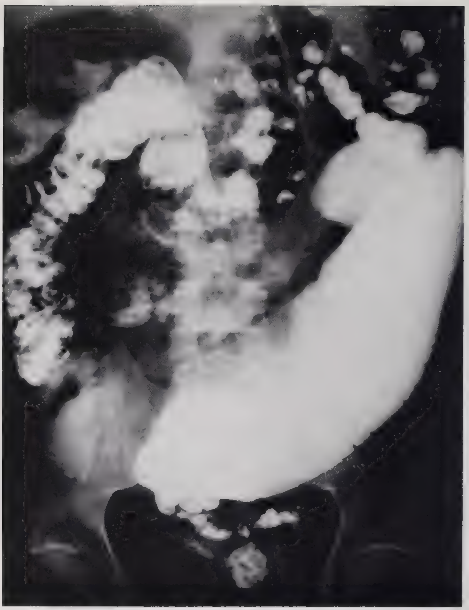

FIG. 1. Study of the small intestine showing the previously bypassed loop of markedly distended proximal small bowel.

loops of small bowel. There was no evidence of free air. Hemogram revealed a hemoglobin of 6.7 gm%, hematocrit 20.7, WBC 6100 per cu mm with a shift to left. Blood chemistries, coagulation studies, urinalysis, chest x-rays, and ECG were within normal limits.

A diagnosis of granulomatous enteritis with exacerbation was made and the patient was treated for partial small bowel obstruction with intravenous fluids, blood transfusions and antibiotics. Barium studies of the small bowel showed a large blind loop which represented the previously bypassed segment of diseased bowel (Fig. 1). Despite therapy, the patient experienced shaking chills and temperature elevation to 104.7°F. Liver and Gallium scans and multiple blood cultures were negative. With the exception of moderate tenderness and guarding over the left lower quadrant, the abdominal examination remained unremarkable. Despite intensive antibiotic therapy, the patient continued to have daily temperature spikes and his condition deteriorated.

Laparotomy was performed on February 8, 1975, and revealed generalized peritonitis with a large amount of purulent ascites. Examination revealed a small perforation and an area of marked induration in the severely diseased previously bypassed segment of bowel suggesting malignancy (Figs. 2–4). The bypassed loop including the previous side-to-side anastomotic site was resected and small bowel continuity was restored by end-to-end anastomosis of grossly normal appearing segments of bowel. The abdominal cavity was extensively drained.

The gross specimen (Figs. 2–4) revealed marked distention and hypertrophy, ulceration and cobblestoning, and a 2-cm area of stony hard induration.

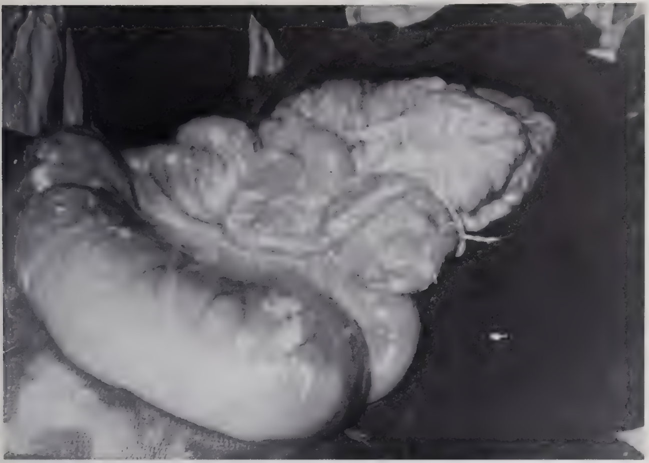

FIG. 2. Operative finding of the bypassed loop. Note the marked distension and thickened wall.

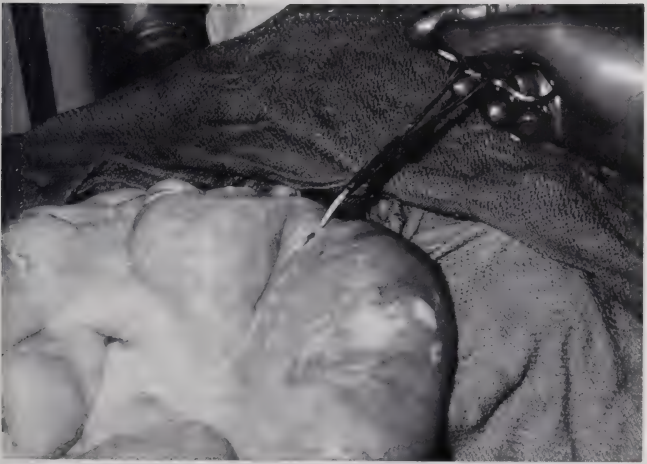

FIG. 3. The area of perforation is indicated by a hemostatic clamp.

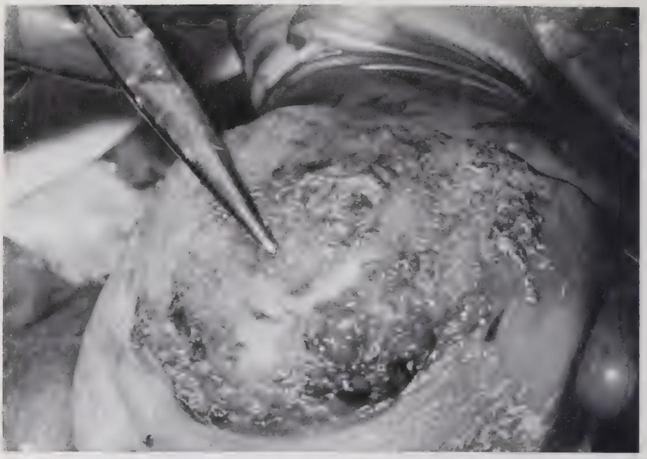

FIG. 4. The partially opened specimen showing typical changes of Crohn's disease.

Pathologic examination showed chronic granulomatous disease of the bowel with an area of well-differentiated adenocarcinoma and metastatic disease to one lymph node (MSH #752342) (Fig. 5). It should be noted that the perforation was not through the area of the carcinoma, but rather through an area of ulceration.

The postoperative course was unremarkable until the eighth postoperative day when the patient developed severe left upper quadrant abdominal pain followed by fever of 102.7°. Examination showed marked tenderness and guarding in the left upper quadrant. Nasogastric suction, intravenous fluids and parenteral antibiotics were reinstituted without improvement in abdominal findings. An anastomotic leak was demonstrated with a gastrograffin study.

Emergency laparotomy was performed on February 24, 1975. There was a partial disruption of the anastomosis with generalized peritonitis. The anastomotic site was exteriorized and converted to a double-barreled jejunostomy. The patient was managed postoperatively with intravenous hyperalimentation, jejunostomy care and systemic antibiotics. With maturation of the jejunostomy stoma, the hyperalimentation was supplemented with jejunostomy feeding. The wound healed per primum and on April 1, 1975, the jejunostomy was closed. The postoperative course was uneventful and the patient was discharged on April 1, 1975, free of abdominal complaints and having well-formed stools. He was readmitted to the hospital with pathologically confirmed diffuse hepatic metastases in June of 1975.

Comment

Twenty-nine cases of carcinoma of the small intestine associated with Crohn's disease have been reported in the literature (1, 3). The case reported here represents the tenth case of carcinoma arising in a segment of small bowel previously bypassed for Crohn's disease (5). The incidence of primary carcinoma of the small bowel is extremely low (4–6), but recent reports suggest

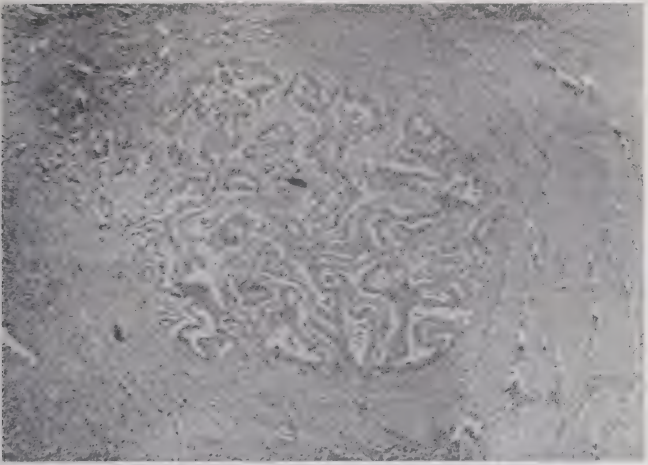

FIG. 5. Microscopic section of the bowel showing an area of well-differentiated carcinoma (100×).

that an association between chronic inflammatory bowel disease (Crohn's disease) and carcinoma may exist (1, 3, 4, 6, 7). The rapidity of transit of potentially carcinogenic substances in the small intestine, the relative sterility of the small bowel compared with the colon, and the high concentration of immunoglobulin A in the small intestine have been suggested as possible factors protecting in the small bowel against the development of carcinoma (5, 8, 9). If these factors are truly significant, then the chronically inflamed intestine of Crohn's disease lacks in such "protective factors" and may be rendered more susceptible to the development of neoplasia. This may be even more so for a bypassed segment of severely diseased bowel left in situ over a long period of time.

The review of literature shows that the average period between a bypass procedure and the discovery of carcinoma is approximately eight years, and carcinoma develops in patients who have had Crohn's disease for an average of 14 years. The average age at which carcinoma develops in Crohn's disease is 42 years, which is about 12 years younger than the average age at which carcinoma of the small bowel develops in the general population (2, 6).

Symptoms related to carcinoma in the small bowel complicating chronic Crohn's disease are nonspecific and are difficult to differentiate from the symptoms of acute exacerbation of quiescent disease. Rapid deterioration of general condition, abdominal mass, perforation, and marked anemia in a patient who has had a long quiescent period should arouse suspicion of complicating carcinoma (7).

The prognosis for carcinoma of the small bowel associated with Crohn's disease is especially poor, with no reported case of a five-year survival. Difficulty and delay in diagnosis appear to be responsible for the poor prognosis.

In general, resection is a better surgical procedure than the bypass procedure in the surgical management of complicated Crohn's disease of the bowel. If an association between the long-standing Crohn's disease and neoplasm can be established, bypass procedure cannot be recommended in the management of Crohn's disease except as a temporary procedure. The threat of the development of carcinoma would become another indication for surgical resection of the involved segment of Crohn's disease.

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Pyoderma Gangrenosum in Ulcerative Colitis: Prevention of the Gangrenous Component

I. M. GELERNT, M.D., AND I. KREEL, M.D.

Pyoderma gangrenosum is one of the uncommon but disabling extra-colonic manifestations of ulcerative colitis. The reported incidence is approximately three percent (1). Grossly, the lesion is characterized by a pink, raised, tender skin lesion. The central portion of the lesion becomes violaceous, and then proceeds to full-thickness necrosis of skin, with undermining of the adjacent inflamed margins. The lesion is extremely difficult to treat once necrosis has occurred. One can expect months of pain and non-healing. Attempts to accelerate healing by the use of skin grafts almost always fail. Often resolution is achieved only following colectomy.

The histologic appearance of the pyoderma lesion suggested to Rostenberg (2) that the lesion is akin to the Shwartzman phenomenon. Several years later Goldgraber and Kirsner (3) described a patient with ulcerative colitis and diffuse necrotic skin lesions, and confirmed the microscopic picture of platelet thrombi and endothelioid proliferation in small blood vessels. It is of interest that this microscopic lesion is not unlike that seen in the arteriolar endothelium in the rejecting renal transplant (4).

It has been known for some time that the dermal Shwartzman phenomenon could be prevented with heparin anticoagulation (5). However, the diffuse colonic ulceration would make heparin an unsuitable agent for treating pyoderma gangrenosum in ulcerative colitis.

Cyproheptadine (Periatin®, Merck Sharp & Dohme) has been shown to be one of the most potent and least toxic platelet deaggregators. It has the unique characteristic of inhibiting both the primary and secondary waves of platelet aggregation without affecting clot retraction (6). The effect is rapid. The drug can be given orally, and has a significant affinity for the platelet membrane. This drug also has a marked antiserotonin and antihistaminic activity. Previous encouraging experience with this drug in inhibiting the vascular phase of the rejection phenomenon in kidney transplantation (7) suggested that it would be useful in the management of pyoderma gangrenosum.

Case Reports

Case 1: A 15-year-old boy with a 9-year history of ulcerative colitis had diffuse pyoderma gangrenosum lesions on both lower limbs, trunk, and left arm (Fig. 1). Within 24 hours of onset, several lesions had already become gangrenous, and others were ecchymotic with gangrene im-

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minent. The patient had been receiving 15 mg of prednisone daily until the appearance of the pyoderma lesions, when prednisone was raised to 30 mg. Colonic symptoms were minimal. Cyproheptadine 4 mg. 4 times daily was started immediately after admission to the hospital. No lesion not already gangrenous progressed to necrosis. The erythematous early lesions resolved within 3 days. The ecchymotic lesions cleared over approximately 10 day (Fig. 1). Even the frankly necrotic lesions appeared somewhat cleaner than had been our experience in the past.

Case 2: A 20-year-old woman with an 11-year history of ulcerative colitis came into the Hospital one day following onset of pain in the left pretibial area. The patient had been receiving azulfadine and prednisone. The colonic symptoms had become exacerbated.

At age 16 pyoderma gangrenosum had developed on the right leg and despite increased steroid dosage had taken approximately eight months to resolve.

The left pretibial lesion was raised and quite tender, with a central purplish hue suggesting beginning necrosis. Twelve hours following the first dose of cyproheptadine the lesion was no longer painful. The discoloration disappeared quickly and six days later the lesion was barely visible.

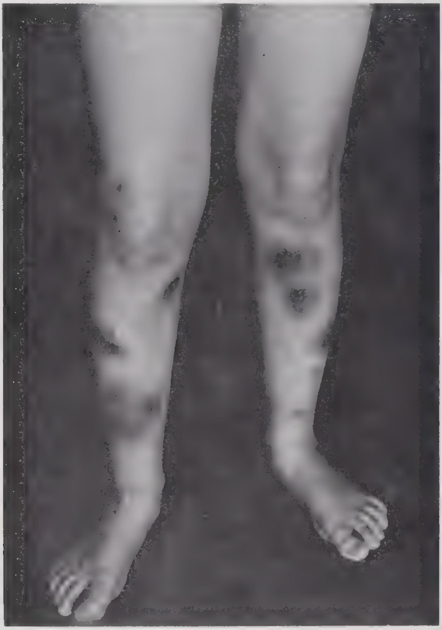

Fig. 1. Pyoderma lesions upon admission to hospital.

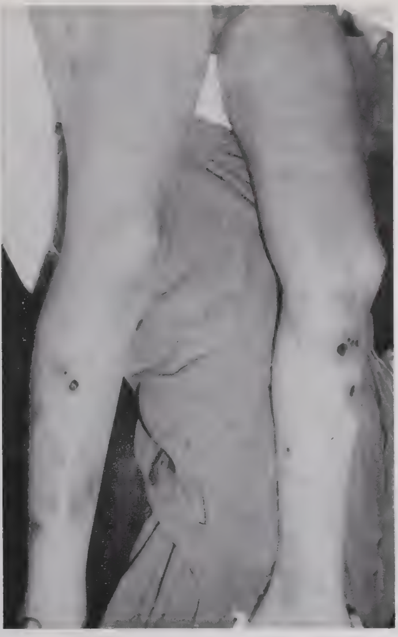

FIG. 2. Seven days following start of cyproheptadine.

Discussion

Cyproheptadine's ability to prevent platelet thrombi and the hyperacute rejection of the heterologous kidney transplant has been demonstrated (7). The dermal Shwartzman lesion in rabbits can be altered with cyproheptadine (8). The phase of gangrenous necrosis is eliminated and the resolution of the local dermal lesion is rapid.

The long duration of nonhealing in the established necrotic, ulcerated pyoderma lesion can be prevented if the ischemic phase of the lesion is bypassed. Early administration of cyproheptadine prevents platelet thrombi in vessels probably having endothelial damage on an immunologic basis. The vasospasm secondary to serotonin release from damaged platelets is eliminated. The rapid improvement in the local lesion merely requires resolution of the inflammatory process, since no tissue necrosis has occurred. Corticosteroids or ACTH in combination with cyproheptadine combat both elements of the problem.

Cyproheptadine as a platelet deaggregator is of special value in a disease characterized by frequent rectal bleeding, since the drug does not produce a bleeding diathesis. The side effects include mild sedation and perhaps some stimulation of appetite which is often of value in these patients. There is no evidence that this drug possesses any immunosuppressive activity.

It is of interest to speculate that the improvement seen in some patients with pyoderma gangrenosum started on large doses of corticosteroids is due in part to the known platelet deaggregating effect of corticosteroids.

Summary

The gangrenous phase of the pyoderma lesion in ulcerative colitis can be prevented with early administration of cyproheptadine (Periactin®). The lesion will progress to early resolution if the ischemic ulcer is avoided. Two patients demonstrating this response are presented.

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Adventitial Cyst of the Popliteal Artery with Secondary Inflammatory Entrapment

LESTER BLUM, M.D. AND FABIO GIRON, M.D.

A case of adventitial cyst of the popliteal artery is presented because of an ancillary entrapment caused by an enveloping scar tissue reaction. The operative management was significantly aided by the use of repetitive arteriography during the procedure.

Since arterial adventitial cysts constitute a comparatively rare but disabling condition in young adults, any additional information throwing light on the behavior of the disease is worth reporting.

In this case, the lesion located in the upper popliteal artery was enveloped by a fascial reaction producing, additionally, an entrapment syndrome. The use of repetitive operative angiography clarified the morbid anatomy and facilitated technical management.

Case Report

R. M. W., a 34-year-old man, was admitted to The Mount Sinai Hospital with a complaint of increasing claudication of the right leg. The onset was gradual but during the preceding two months, the claudication had become progressively more severe. Walking was reduced to one block before the onset of pain. Tennis was impossible. There was no rest pain, no paresthesias, and the left lower limb was asymptomatic.

Examination revealed a healthy adult. Both lower extremities were equal without any trophic changes. All pulses were palpable in both extremities; however, the oscillations below the right knee were distinctly diminished. In addition, the right pedal pulses disappeared after exercise. Extreme flexion of the knee did not obliterate the pulse. A right femoral arteriogram (Fig. 1) showed a curvilinear stenosis of the popliteal artery ("scimitar sign") just above the level of the femoral condyles. The distal arterial tree was normal. A preoperative diagnosis of adventitial cyst of the popliteal artery was made.

At operation the patient was placed in a lateral decubitus position to permit medial and posterior approaches to the popliteal artery. The course of the popliteal artery was free of the popliteus tendon and the medial head of the gastrocnemius muscle. However, it was found bound down to the posterior aspect of the capsule of the knee joint by a tough fascia-like envelope of connective tissue. After the artery was delivered an adventitial cyst located on the posterior medial aspect of the vessel was found. The lesion presented as bluish dilatation involving only half of the arterial circumference over a length of three centimeters (Fig. 2).

The release of the enveloping tissue resulted in a moderate increase in pulsation distal to the lesion. For this reason, before incising the cyst, an arteriogram was made. The film (Fig. 3) showed a persistence of the asymmetrical filling defect in the artery, obviously, due entirely to the cyst since the fascial entrapment had been released. Incision and unroofing of the cyst wall yielded four ml of a viscous crystalline material. A repeat intraoperative arteriogram (Fig. 4) showed a complete restoration of the lumen and contour of the popliteal artery with no evidence of intrinsic

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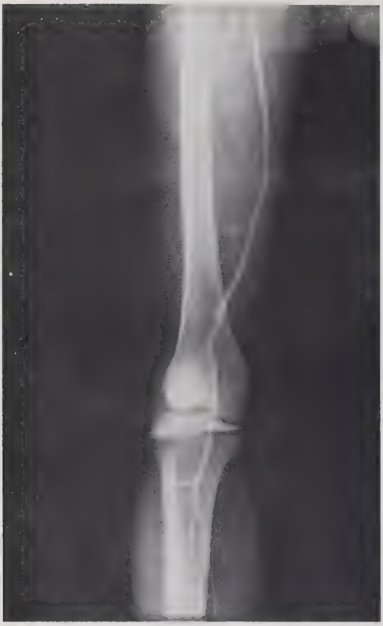

FIG. 1. Preoperative femoral arteriogram. Note "scimitar sign."

arterial disease. The patient enjoyed an uneventful convalescence and was discharged 12 days postoperatively. At six weeks he had resumed normal activity, including playing tennis.

Discussion

Since the original description by Ejrup and Hiertonn (1), about 45 cases of cystic adventitial disease of the popliteal artery have been reported. The clinical manifestations are, characteristically, unilateral intermittent claudication of sudden onset in a relatively young male, as described in several collective reviews (2–4). The pulses in the foot can be made to disappear by extreme flexion of the knee joint (Ishikawa sign) (5). The arteriography shows, typically, a localized asymmetric narrowing of the artery in the region of the femoral condyles. The entity has been described in other locations (6).

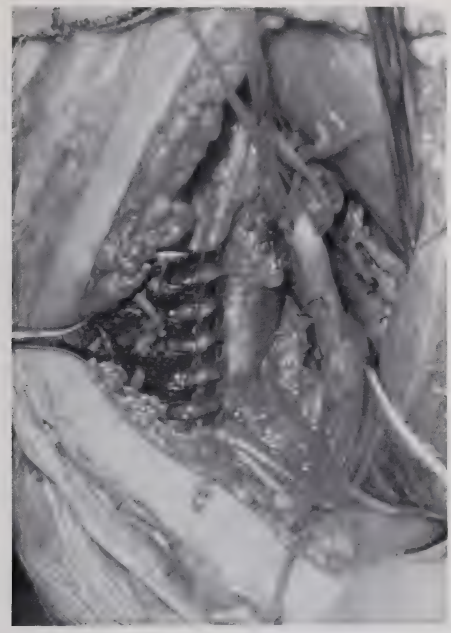

FIG. 2. Operative appearance of lesion.

The etiology of this lesion has not been established. Although a communication between the adventitial cyst and the synovial cavity of the knee joint has been postulated (7), the theory lacks sufficient documentation. On the other hand, the possibility of inclusion within the adventitia of the artery of mucin-producing cells can be accepted even without demonstration of a knee joint communication. As in ganglions, or Baker's cyst in the same location, the communication with the joint is not always observed (8).

The unusual type of entrapment of the popliteal artery present in our case makes the traumatic implantation of synovial cells within the adventitia of the artery a very acceptable explanation. The thick fascial envelope found around the artery probably represents an inflammatory reaction to trauma, since no evidence of systemic connective tissue disorder appeared in this patient.

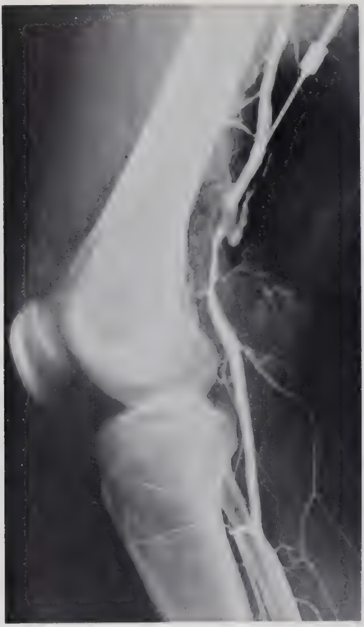

FIG. 3. Arteriogram following operative release of inflammatory entrapment with adventitial cyst still in place.

In about half of the cases described in the literature a segmental replacement of the popliteal artery using saphenous vein or prosthetic material was employed. In nineteen patients (9) simple evacuation and partial excision of the cyst was carried out with good results. In only two cases the cyst recurred but it was easy to reoperate and again evacuate the cyst (9). In dealing with a young population it would appear that preservation of the artery is the method of choice, since the long-term fate of saphenous vein or prosthetic material in the popliteal area is not entirely known.

We believe that the use of intraoperative arteriograms is an essential requirement to avoid unnecessary graft replacement. We see no reason to replace the popliteal artery if a normal lumen is reestablished after evacuation of the cyst and the gross anatomy of the area shows no obvious destruction of the remainder of the arterial wall.

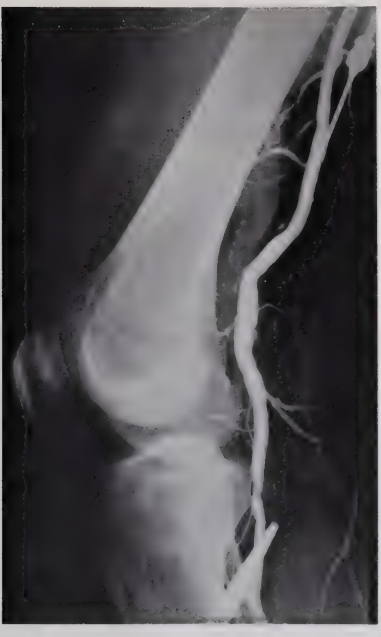

FIG. 4. Arteriogram following excision of adventitial cyst.

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An Interesting Case of Cancer of the Sigmoid with Concomitant Cancer in an Anal Fistula

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Carcinoma within an anal fistula is frequently thought of, but there are only seventy-four documented cases in the literature. A fistula in ano is a chronic suppurating tract with a variable amount of discharge. The fistula may arise from a superficial ano-rectal abscess, an ischio-rectal abscess, pelvi-rectal or, occasionally, a retro-rectal abscess. A pelvi-rectal abscess forms in the cellular tissue between the pelvic peritoneum above and the levator ani muscle below. This abscess may arise primarily from pelvic inflammatory disease in the female, prostatitis in the male, appendicitis, colonic diverticulitis and, as in this case, from a perforated sigmoid carcinoma with abscess formation

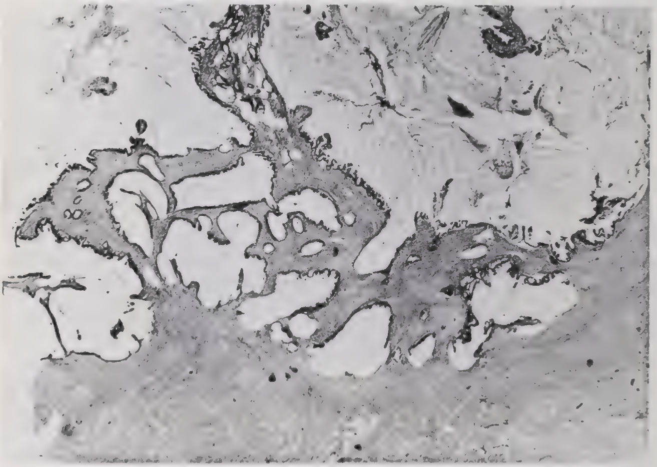

FIG. 1. Anal fistula (40 ×).

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along the left lateral pelvic peritoneum, resulting in a pelvi-rectal fistula. The abscess tracks down through the levator ani muscles, infecting the ischio-rectal space and points externally.

Other causes of adenocarcinoma in a fistula are:

1. Malignant change of small duplications of bowel;
2. Malignant change of fistula itself, usually squamous although possibly adenocarcinoma;
3. Malignant change of intramuscular glands;
4. Breakdown of a primary anal canal neoplasm;
5. Spread of cancer cells from a growth higher in the lumen of the bowel implanted as a secondary deposit

Case Report

A 47-year-old blind man had a draining anal fistula of about three months duration. This was removed by a routine fistulectomy. Pathologic report was fistula in ano infiltrated with adenocarcinoma (Fig. 1).

Procto-sigmoidoscopy was negative for 24 cms. Barium enema showed complete obstruction to the retrograde flow of barium (Fig. 2). The preoperative diagnosis was cancer of recto-sigmoid with

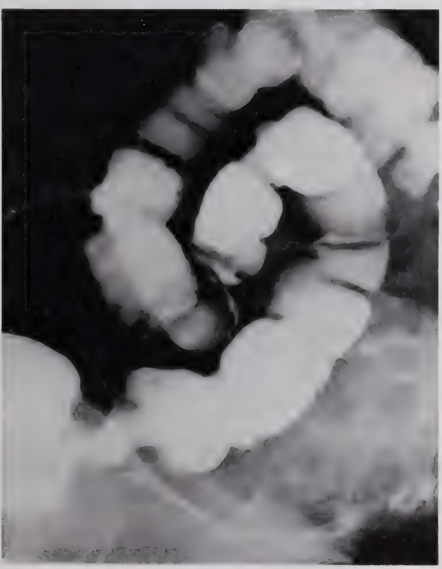

FIG. 2. X-ray of the recto-sigmoid showing the anular carcinoma.

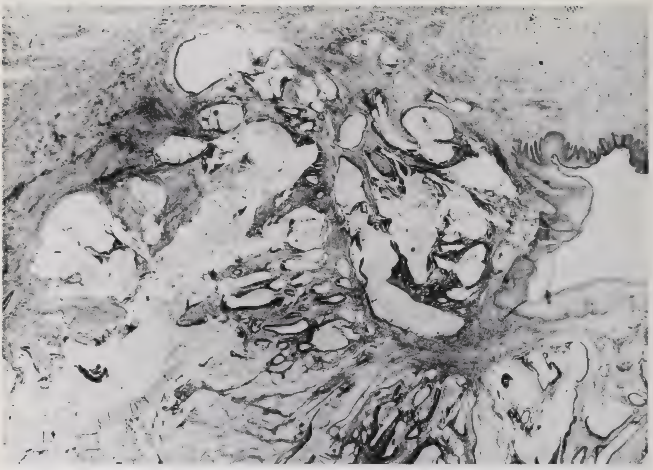

FIG. 3. Carcinoma of the rectosigmoid (40 ×).

local metastases. A combined abdomino-perineal resection of the recto-sigmoid colon containing a perforated tumor with abscess formation in the left lateral pelvic peritoneum plus a wide resection of perineal tissue was performed three weeks after the fistulectomy.

The pathologic report was adenocarcinoma of sigmoid colon with perforation and infiltration through to the perineal tissue (Fig. 3).

After a stormy postoperative course, caused by a reflex ileus, the patient was discharged with the wound healed and the colostomy functioning well.

This case presented initially as a fistula in ano. The pathogenesis of this fistula was most likely the perforation of the cancer of the recto-sigmoid tracking down as an infection. The ultimate prognosis is poor, although the patient has returned to work. When last seen, eighteen months postoperatively, the patient was well with no evidence of metastatic disease. Although he was blind, he was able to manage his colostomy.

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Manager Stress in a Hospital Environment: A Case Study

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Abstract

Through the use of traditional statistical techniques and the Extensor System, it is possible to identify, rapidly and with statistical precision, a large number of variables that define and describe the work process. This paper examines *one* use of such a data base: the identification and assessment of stress in an organization.

The findings indicate that there is considerable commonality across individuals in their reaction to stress. The identification of these common reactions makes it possible to deal with stress on the organizational level, by applying appropriate procedures for coping, avoidance, or substitution among the participants in the study.

DESIGN OF STUDY

Seven executives of a midwestern hospital decided to examine the sources of stress in their daily work activities. In order to do so the following was done:

1. *A complete enumeration of all activities:* This enumeration consisted of categories that were well defined and mutually exclusive, and that applied uniformly to all participants.

2. *Choosing judgment dimensions related to stress:* In addition to the enumeration and categorization of *activities*, several other dimensions were chosen for study as well. These dimensions addressed areas of behavior, judgment and attitude that conceivably could be related to a sense of stress in the individual participating in the study. An exact specification of these dimensions is given below.

3. *Random sampling:* A random sampling procedure was devised in order to collect the information sought in the study.

4. *Identification of stress factors:* The analysis of the findings had to be constructed in such a fashion that common, specific stress-producing events could be identified, quantified, and dealt with by the participants.

METHODOLOGY

Instrumental Activity Dimension: In order to measure the *instrumental activities* for the participants, one must produce a list of categories that cover

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all possible and relevant work-related instrumental behaviors. The list agreed upon by the participants is reproduced below:

Instrumental Activity Dimension (Abbreviations in Parentheses)

1. Talk, meet on a one-to-one basis (Talk, meet 1:1)
2. Talk, meet with more than one, but less than five (Talk, meet 4 or less)
3. Talk, meet with five or more (Talk, meet 5 or more)
4. Incoming telephone calls (Telephone IN)
5. Outgoing telephone calls (Telephone OUT)
6. Write, dictate, punch Extensor Unit (Write, dictate, punch)
7. Think
8. Read
9. Study
10. Edit/sign
11. Sort, file, retrieve
12. Other

In the subsequent regression analysis, one of the categories listed above had to be omitted. When the amount of time spent on *all* activities is known, *and* the amount of time spent on *11* activities is known, the time spent on the twelfth activity is easily derived. Thus, adding a twelfth category to the analysis adds no new information. Thus, 11 categories are used. To avoid computer problems that would arise if superflous information was used, the twelfth category, "Other," was consequently omitted from the analysis.

Contact Dimension: The amount of contact that an executive experiences with his superiors, colleagues and subordinates may be related to his sense of stress. But since this study directs itself to stress factors held in common by *all* participants, one can only examine those interactions that are common to all participants. There were six of these common interaction categories:

1. Self
2. Management Group (consisting of all participants)
3. Secretary
4. Health Agency/Professional Association/Government
5. Consultant/Advisor/Vendor
6. Other

Since a number of individualized categories had to be omitted from the analysis, there was no need to omit any of the common categories listed above to avoid the problem of duplicating information.

Initiative Dimension: Conceivably an individual may be experience stress differently when his work activity is initiated by himself and when it is initiated by someone else. The relationship of initiative to stress can be examined if we add another dimension:

Own initiative
Other initiative

The sum of these must equal 100 percent. Thus, "Own initiative" is included in the analysis as a variable.

Planning Dimension: Much executive work is planned in advance, but much is unplanned, and consists of day-to-day activities that cannot easily be anticipated. To examine if planning affects stress, the following dimension was added:

Planned
Unplanned

"Planned" was included in the analysis as a variable.

Delegation Dimension: Over the years, many executives have accumulated tasks and duties that may have little relevance to their original job descriptions. It is reasonable to assume that if an executive judges many of these tasks to be unnecessary or inappropriate, their presence might affect his sense of stress. The following scale was constructed:

Delegate Now = 2
Delegate Maybe = 1
Don't Delegate = 0

The higher the score on this dimension, the more likely it is that the task is inappropriate or unnecessary for the executives measured in the study.

Functional Dimension: Ideally one should be able to translate each executive's job description into a dimension that consists of all the tasks and processes that characterize the functional areas to which his instrumental job activities are applied. In reviewing this dimension for each executive, the following categories were found to be common to all participants:

Finance
Educational Programs
Planning for Meetings
Other

Management Role Dimension: When an executive operates as a manager, there is a limited set of categories that describes *managerial* (as opposed to *specialist*) behavior. The categorization generally depends on what management school or philosophy one subscribes to, but the following code enjoys considerable popularity in the field of management*:

1. Planning
2. Investigating
3. Coordinating
4. Evaluating
5. Supervising
6. Staffing
7. Negotiating
8. Representing
9. Educating
10. Developing Constituencies
11. Personal

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* See Mahoney, T. A.: The Jobs of Management, Industrial Relations 4:97–110, 1965; and David, S. and Henshaw, S.: *Decision Analysis in Hospital Administration.* Association of University Program in Hospital Administration, Washington, D.C., April 1974.

The category of "Other" was omitted. The definitions of the categories are supplied in Appendix A.

Productivity Dimension: In discussions with the executives, it was pointed out that they often felt productive doing tasks that might not have a direct relationship to the Planned/Unplanned Dimension, or to certain physical activities. If this sense of *productivity*, which is a subjective measure, had any relationship to stress, it might provide valuable clues for effective staff development. Consequently, a "Sense of productivity" variable was included:

Feeling productive
Feeling nonproductive

"Feeling productive" was included in the analysis.

Manager Role Dimension: There are situations when a manager operates as a specialist, rather than as a manager. This is the situation if a corporate treasurer executes simple bookkeeping tasks, or if the president chooses to teach a class in a discipline that he masters. Presumably doing such activities may be putting the executive in a realm where he has supreme skills and feels professionally adequate—or, his skills may have deteriorated for lack of use over time. In either case, the *role* is likely to be correlated with stress.

Data Collection Procedures: The study was performed using Extensor Units over a period of six weeks (including one week of test measurement). The Extensor Unit is a device that consists of three elements:

1. A random impulse generator, that emits signals at random intervals (30 times during an 8-hour day), by sound and by light.
2. A code list for classifying behavior, attitudes, and judgment (the code list consists of the dimensions enumerated above, plus a dimension of stress, using "stressed/nonstressed" as categories).
3. A means for storing data in machine-readable format (in the model used in this experiment, a punchcard was used for this purpose).

Each participant responded to the unit each time the signal sounded, and entered information onto the card for all the dimensions of the study. Over the period of the study this produced a total of 5800 observations in 10 dimensions, yielding a total of 58,000 randomly collected peieces of information (5800 × 10). Procedures were devised to handle absences from the office, and the final data base represented a substantial achievement in activity sampling in terms of the detail and precision of the data.

ANALYSIS OF THE FINDINGS

Distribution of Stress in the Organization: Looking at the group as a whole, it can be shown that the group registered stress 8 percent of the time (unweighted average across participants), whereas 92 percent of the time was characterized as "relaxed." The variation across executives was interesting: the most stressed executive was stressed 23 percent of the time, the least stressed executive was stressed .4 percent of the time. Clearly the stress in the organization was unevenly distributed. The first question that arises is whether there are common factors contributing to stress across all individuals. If so, what are these common factors?

Relationship between Stress and Other Variables: In order to relate stress to other relevant variables, a correlation analysis was performed on the data. The zero-order correlation coefficients with stress, can be divided into positive and negative stress correlates. A positively correlated variable is one which is associated with increased stress. A negatively correlated variable is one which is associated with reduced stress.

Positive Stress Correlations	
Unplanned	.237
Edit/Sign	.141
Think	.126
Negotiating	.122
Educating	.082
Personal	.080
Finance	.058

Note that unplanned occurrences show the single greatest correlation with stress in the organization. With a correlation coefficient of .234, approximately 5.5 percent of the total stress is explained by this factor ($.234^2 = 5.5$).

For the executive group as a whole, 80 percent of the time is planned—only 20 percent is unplanned. Conversely, planned activities act as a factor in reducing stress. Conceivably, the nature of the unplanned activities account for this finding and will be examined in greater detail later.

The editing and signing activity shows the second greatest correlation with stress. This is often done under considerable time pressure.

Thinking as an activity is perceived to be stressful. The temptation *not to think* may thus have a rationale; it relieves temporarily a sense of stress. Stress-reducing exercises (such as yoga and transcendental meditation) consciously aim at the elimination of ordinary thinking activity.

It is not surprising that the process of negotiating is perceived to be stressful—it frequently takes on the character of an adversary relationship. The other correlations (educating, personal, finance) are so small that their practical significance is questionable.

Negative Stress Correlations	
Acting as a manager	–.197
Talking—Meet 1:1	–.111
Sense of being productive	–.087
Meeting/Planning	–.079
Self	–.078
Write, Dictate, Punch	–.077
Evaluating	–.070
Coordinating	–.063
Investigating	–.054

It is interesting to note that acting as a manager is a stress reducer. Conversely, specialist functions thus seem to impose a burden on the manager.

Meeting on a one-to-one basis reduces tension; and 28.2 percent of total time is spent on face-to-face encounters. This is more than for meetings with 3-4 people (11.7 percent) or 5 or more (26.3 percent). The stress-reducing quality of the one-to-one encounters might well explain the frequency of such encounters.

On the average, when the managers feel productive, they do not feel stressed. This is the most interesting finding. It indicates that productive workers are likely to be less stressed than unproductive workers. Increased productivity may thus serve as a stress reducer. The underlying rationale for this reasoning is the assumption that the subjective feelings of productivity are closely related to the realities of organizational productivity.

Planning for meetings, being alone, writing, and performing management functions such as evaluating, coordinating and investigating are all stress reducing, but of little statistical significance.

Dealing with Stress: It should be kept in mind that there are several ways of dealing with stress, if it is deemed to be desirable to do so. One may:

1. Avoid stress-producing activities
2. Learn to cope with the unavoidable stress
3. Substitute stress-reducing activities

A chief executive may delegate negotiation activities to his vice-president (avoiding stress), or sign up for a training session in negotiating (coping with stress). He may also restructure the negotiating process, drawing on other resources and techniques that he favors over the current process (substitute stress-reducing activities). Whatever method is chosen, knowledge of the sources of his stress provides a rational basis for dealing with executive stress.

Multivariate Analysis: Note that correlation coefficients neither indicate a cause and effect relationship (they measure *associations*) nor do they correct for the effect of other variables that may exert influence simultaneously on the paired comparisons. In order to correct for the effect of such influences, a multivariate, linear regression analysis was performed on the data, using stress as the independent variable. The results are discussed below.

A multivariate, linear step-wise regression was run on the University of Minnesota UMST 580 Computer Program, using the CDC Cyber 7000 computer. A total of 37 independent variables were run against stress as a dependent variable, using dummy variable coding for nonscaled variables. Excluding nonsignificant variables by the F-test (significance level .95), the final equation contained 24 variables. The R^2 was .229, the standard error of estimate .287, and the constant was .611.

A number of ways may be used to present regression results in a comprehensible manner. Perhaps the most acceptable interpretation is to view stress as the net result of positive and negative forces. Using the total, weighted average of stress between participants (12.1 percent of total time), it is possible to express this figure as the difference between the sum of all terms in the regression equation where the average of each variable has been multiplied by the corresponding regression coefficient, plus the constant.

The reason for this procedure is the need to represent fairly the amount of stress over time in the organization. Stress can be measured both by intensity and duration. For example, the process of negotiations may cause much stress while it lasts (regression coefficient is +.25), but the total amount of time spent on negotiations may be a small fraction (actually 4 percent of the time). The table below takes both of these infleunces into account, and shows the product of stress intensity (the regression coefficient) and stress duration (the average amount of time consumed by the variable). "Negotiations" are thus found to add less than one percent to total stress, while meetings on a face-to-face basis (which is stress reducing with a regression coefficient of −.42) reduces stress by 10.53 percent due to their frequency and time consuming nature (about 25 percent of the time). A listing of the stress intensities and the average duration is also found in Table I.

TABLE I

Stress Intensities and Average Duration

Stress Increasers	Stress Intensity (Regression Coefficient)	Average Duration (Average Value)	100 × Total Effect
Unplanned	.133	.231	3.07%
Planning	.063	.217	1.37%
Negotiating	.246	.040	.98%
Education	.138	.039	.54%
Supervising	.055	.059	.32%
Staffing	.080	.029	.32%
Constant	—	—	61.07%
		Total	67.58%
Stress Reducers			
Talk, Meet 1:1	−.421	.250	−10.53%
Manager	−.133	.702	−9.34%
Feeling Productive	−.051	1.754	−8.95%
Talk, Meet, 5 or more	−.302	.256	−7.73%
Self	−.223	.236	−5.26%
Talk, Meet, 4 or more	−.345	.113	−3.90%
Write, Dictate, Punch	−.223	.122	−2.72%
Telephone, OUT	−.334	.033	−1.10%
Telephone, IN	−.375	.027	−1.01%
Educational Program	−.118	.081	−.96%
Hlth. Agency/Profess. Assn./Gov't.	−.054	.154	−.83%
Study	−.163	.046	−.75%
Read	−.136	.041	−.56%
Planning Meetings	−.065	.085	−.55%
Consultant/Adv./Vendor	−.081	.052	−.42%
Other	−.078	.048	−.37%
Finance	−.066	.043	−.28%
Sort/File/Retrieve	−.111	.022	−.24%
		Total	−55.50%
Stress Increasers	67.58%		
Stress Reducers	55.50%		
Net Stress	12.08%		

Interpretation of the Findings: There is a large constant among the "Stress Increasers"—61.07 percent. This represents the "noise" in the regression, it is the stress-producing effect of all the variables that are not explicitly present in the regression.

Note also the stress-inducing effect of exercising the management role. It is somewhat paradoxical that unplanned events are a chief source of stress, whereas the planning process itself is stressful, yet less so than the occurrence of unplanned events.

Note also that *all* meeting activity reduces stress: most when it is on a one-to-one basis, less so when it is five or more participants, and least when the meeting has three or four participants. It is clear that the stress-reducing effect of meetings is a powerful incentive to continue meeting activities, particularly on a face-to-face basis.

Acting as a manager rather than as a specialist is stress reducing, although exercising certain managerial functions is stress increasing. But controlling for these functions, there is a net benefit (in terms of stress reduction) in being a manager. "Feeling productive" is another significant stress reducer. This clearly indicates that people may strive to be productive for reasons other than financial; it may be an effective way to reduce tension.

It is interesting to note that when all other variables are controlled, telephone activity, whether it is initiated internally or externally, is stress reducing.

The relatively low R^2 indicates that the common variables regression has explained only 23 percent of all the stress across individuals in the organization. But this is a rather remarkable achievement. It was unexpected that such commonality could exist across individuals, and that so many influences could be identified as statistically significant variables in explaining organizational stress.

Clearly there are individual variations that are unique to each executive. In other words, while most executives are stressed by a core of common influences, there are bound to be influences that remain unique to each individual.

Such influences can indeed be found, and they spotlight the ability of certain individuals to handle activities that would be most stressful to others. The R^2 also increases when individual regressions are run, indicating a better specification of the stress problem when purely individual variables are used.

It is a profound experience to view oneself from the perspective of a *personal* stress regression. One can compare the organizational findings with one's own. The individual-specific relationships to stress are likely to show idiosyncratic deviations from the organizational findings, adding insight about individual differences in their reactions to stress.

Thus, individualized regressions may point towards actions that could relieve stress and tension for the participant. Once an individual decides to reorganize his work activities, it becomes possible to estimate, using the individualized regression equation, the degree to which his sense of stress will be changed and the direction of this change. This opens up a remarkable opportunity to experiment with changes *on paper*, and predict outcomes without engaging in human experimentation. What is possible to do for individuals, can be done for organizations as well.

It should be emphasized that several variables were found *not* to be stress inducing or reducing in this study. It did not seem to make a difference whether a task was initiated by oneself or by somebody else or whether the task was judged to be delegable or not.

All four of the functional dimensions were found to be stress reducing, indicating that shared functional tasks are probably less stressful than tasks for which there is unique individual responsibility. Nine of the eleven instrumental activity dimensions were found to be stress reducing, indicating that the controlled effect of physical activity per se reduces stress. This comes as no surprise, considering the established view that physical activity in general is assumed to have a beneficial effect on mental health, particularly if it is the activity itself that dominates the picture to the exclusion of any ulterior motive or purpose. Three of the six contact dimensions emerged as significant stress reducers, indicating that human interaction seems, on the average, to exert a benign influence on a sense of stress. Only the five management role dimensions of planning, negotiating, educating, supervising, and staffing (of a total of eleven) were found to be uniformly stressful, yet outweighed by the controlled effect of functioning as a manager in general.

The process of negotiation is by far the single most stressful event (coefficient: .246). "Educating" comes next with .138, and "unplanned events" follows with .133. The process of planning increases stress slightly (.063), but to the extent that this process can forestall unplanned events, it clearly pays to plan in spite of its stress-inducing qualities.

The single greatest stress reducer is face-to-face meetings (–.421), then follows "Telephone IN" (–.375), "Talk, Meet, 4 or Less" (–.345), "Telephone OUT" (–.334), and "Talk, Meet, 5 or more" (×.302). Note that all these events include a necessary interaction with people.

Least stress reducing is "Feeling productive" (–.051) and "Finance" (–.066), neither of which necessarily involves human interaction. The therapeutic effect or social intercourse seems to be pervasive from the point of view of tension reduction.

It should be kept in mind that in changing behavior in order to respond to these findings, one must keep the overall objectives of the organization in mind. In this setting a series of organizational changes were, in fact, made based on findings from the study, but using far more complex criteria than stress reduction. Another paper will discuss these findings in detail. The effect of these changes on stress can be tested by remeasuring after the changes have been implemented. Such measurements could become routine in a sophisticated managerial environment.

Summary

Through the use of traditional statistical techniques and the Extensor System, it is possible to identify, rapidly and with considerable precision, a

large number of variables that define and describe the work process. This paper examined *one* use of such a data base: the identification and assessment of stress in an organization. A large number of other uses can be made of such a data base—some of these will be enumerated in subsequent papers.*

The findings indicate that there is considerable commonality across individuals in their reaction to stress. The revelation of these common reactions makes it possible to deal with stress on the organizational level, by applying appropriate procedures for coping, avoidance or substitution among the participants in the study. From a management standpoint, the most significant finding in this study is that at least for the participants, productivity or a sense of productivity was associated with reduced stress. This would tend to strengthen findings by McGregor and Herzberg dealing with Theory "Y" and hygienic factors in the organization. Certain barriers to productivity can and should be removed through the effective manipulation of time and activities.

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Time Management in a Hospital Environment: A Case Study

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INTRODUCTION

Time Management

The field of time management is not highly developed as an academic discipline. The authors of articles and books in the field tend to focus on the *need* for effective time management, the identification of time wastage, common sense advice on restructuring and reorganizing work, and procedures for analyzing the work process in a time context. Peter F. Drucker has stressed the need for viewing time as the primary resource: "Time is the scarcest resource and unless it is managed, nothing else can be managed" (1).

R. Alec Mackenzie (2) has provided the most comprehensive bibliography on the management of time (112 entries), and has provided an integration of the conceptual frameworks of management theory and time management principles. The classic academic reference still is Sune Carlson's work on executive behavior (3) in which dimensions of executive behavior were defined and measured. On the implementation level, in addition to the work of Mackenzie, F. D. Barrett (4) and Alan Lakein (5) have developed extensive procedures for improving time usage and have applied these concepts to personal and corporate planning. The selective list of contributors discussed above represents a break from the traditional industrial engineering approach to work measurement.

Conventional Work Measurement

Work measurement and work sampling are usually associated with time and motion studies, the development of unit standards for work, and the use of especially trained observers.

There is a two-fold problem with the use of observers: 1) The presence of the observer is likely to affect the behavior of the observed individuals (the Hawthorne Effect (6)); and 2) There are considerable problems in assuring observer reliability. Owen Gilbert (7) estimates that observers may deviate 10 to 20% from the group mean.

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The use of time records, kept by individuals, has several disadvantages: 1) Even simple forms take an inordinate amount of time to complete, interrupt the work process, and create irritation and compliance problems; 2) The data do not exist in a machine-readable format, and the classification and analysis of the data become prohibitively expensive; 3) The vehicle for self-recording is usually a time log, where the sampling procedure must be developed simultaneously in order to make the self-recording a manageable task. Often a participant will enter into the time log what he is doing every 15 minutes. This is likely to create cyclical sampling biases, since the known interval will condition behavior, and cause managers perhaps to engage in long-range planning, or other "desirable" activities every 15 minutes. If the sampling procedure is omitted, and the participants simply write down everything that happens during his working day, the recording task becomes prohibitively onerous.

The Extensor System

A completely new development in the field of time management is the Extensor System. Combining the measurement of attitudes and judgments with random activity sampling, the system can be used in any situation where self-recording is feasible or desirable. The Extensor Unit is a device that consists of three elements:

> A random impulse generator that emits signals at random intervals (30 times during an 8-hour day), by sound and by light.
> A code list for classifying activity, function, attitude and judgment.
> A means for storing data in computer readable format. (The model used in the current study used a punch card for this purpose.)

The Unit was invented and developed by a Swedish firm, System Frekvensor AB, in 1969 and introduced to a variety of uses in Scandinavia and England over the last few years. The Unit became available in the United States in 1974, and was first used on the American continent in a study of hospital managers in Minneapolis during the months of February and March, 1974. Seven Hospital executives decided to use the Extensor System in order to accomplish ten different objectives.

Objectives of the Study

1. Classify, identify, and improve their use of time (Instrumental Activity Dimension)*
2. Determine the nature and amount of interaction and communication within the group (Contact Dimension)
3. Study the originating authority for the initiation of tasks in the hospital (Initiative Dimension)
4. Identify the frequency of unplanned events occurring during the sampling period (Planning Dimension)

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* The "Instrument Activity Dimension" refers to the nomenclature used in the code list, and will be discussed under Methodology.

5. Define and identify the amount of inappropriate work activities by applying a judgment of whether work activities should be performed or delegated (Delegation Dimension)
6. Produce an inventory of job activities for each participant (Functional Dimension)
7. Study the process of management by identifying the frequency of occurrence of various management activities (Management Role Dimension)
8. Produce a subjective evaluation of the productivity of each participant, by asking them to report whether they felt productive or not (Productivity Dimension)
9. Draw a distinction between situations when the participants were operating as managers and specialists (Manager Role Dimension)
10. Measure the amount of stress felt by the participants, and examine the associations between stress and all other variables (Stress Dimension)

All these objectives were achieved on completion of the study.

Procedures

During the study, each participant responded to the Unit each time the signal sounded, and entered information onto the card for all the dimensions of the Study. Because the procedure was simple, it required only a few minutes per day. During the five weeks of measurement, the study produced a total of 5800 observations in ten dimensions, yielding a total of 58,000 randomly collected pieces of information. Procedures were devised to handle absences from the office, and the final data base represented a substantial achievement in activity sampling in terms of the detail and precision of the data.

METHODOLOGY

The Development of the Code List

The key to any successful study of executive behavior is a carefully defined and designed code list. Each of the objectives of the study had to be translated into an appropriate dimension reflecting the objectives. Each dimension must consist of a set of complete and mutually exclusive categories. The code list, its nomenclature, definitions, categories, and use were determined through an intensive process of management group discussions requiring approximately 8 hours. In addition to definitional discussions prior to starting the study, the participants went through a week of test measuring in order to sharpen up the definitions and note any individual interpretational difficulties. The most basic dimension is a description of the physical, or kinetic, activities of the participants. This is called the "instrumental activity dimension." These activities are performed in order to further a *purpose* or a *function*, and are generally not representing goals or ends in and of themselves. In the following, starting with the instrumental activity dimension, each of the ten dimensions will be described and discussed.

Instrumental Activity Dimension

In order to measure the *instrumental activities* for the participants, one must produce a list of categories that covers all possible and relevant work-related instrumental behaviors. The list agreed upon by the participants is reproduced below:

Instrumental Activity Dimension (Abbreviations in Parentheses)

1. Talk, meet on a one-to-one basis (Talk, meet 1:1)
2. Talk, meet with more than one, but less than five (Talk, meet 4 or less)
3. Talk, meet with five or more (Talk, meet 5 or more)
4. Incoming telephone calls (Telephone IN)
5. Outgoing telephone calls (Telephone OUT)
6. Write, dictate, punch Extensor Unit (Write, dictate, punch)
7. Think
8. Read
9. Study
10. Edit/sign
11. Sort, file, retrieve
12. Other

Contact Dimension

The amount of contact that an executive experiences with his superiors, colleagues and subordinates contributes insight about the participant's total work environment. Because different patterns of interaction are expected, the contact dimension was different for each subject, although some of the categories were the same for each participant. Following is a typical contact dimension:

1. Self
2. Management Group
3. Secretary
4. Physician
5. Health Agency/Professional Association
6. Community Agency
7. Board Member
8. Auxiliary
9. Consultant/Advisor/Vendor
10. Educational Institution/Student
11. Patient/Patient's Family
12. Other

Initiative Dimension

A person discovers how much he controls his job, as opposed to others directing him, when the initiator of each task is recorded. The initiative dimension in this code list consisted of:

Own initiative
Other initiative

Planning Dimension

One of the most commonly emphasized functions of a manager is planning. Therefore, this code list contained an indicator of whether each task was:

Planned
Unplanned

Delegation Dimension

Over the years many executives have accumulated tasks and duties that may have little relevance to their original job descriptions. One way of measuring the amount of time which an executive devotes to tasks which he feels others should be doing is through the use of the following dimension:

Delegate Now
Delegate Maybe
Do Not Delegate

Functional Dimension

Ideally one should be able to translate each executive's job description into a dimension that consists of all the tasks and processes that characterizes the *purposes* for which he engages in instrumental job activities. Because this dimension is different for everyone in the Study, one typical functional dimension is shown below:

1. Finance
2. Health Care Delivery
3. Medical Staff Relations
4. Education Program
5. Community Relations
6. Government Regulations
7. Legal/Litigation
8. Research
9. Board of Governors
10. Shared Services
11. Management (Planning Meetings)
12. Other

Management Role Dimension (Appendix A)

When an executive operates as a manager, there is a limited set of categories that describes *management* (as opposed to *specialist*) behavior. The categorization generally depends on the management school or philosophy to which one subscribes, but the following code enjoys considerable popularity in the field of management (8):

1. Planning
2. Investigating
3. Coordinating
4. Evaluating
5. Supervising
6. Staffing
7. Negotiating
8. Representing
9. Educating
10. Developing Constituencies
11. Personal
12. Other

Productivity Dimension

In discussions with the executives, they mentioned that they often felt productive doing tasks that might not have direct relationship to the planned/unplanned dimension, or to certain physical activities. Knowledge of this *sense of productivity* which is a subjective measure, may provide valuable clues for effective staff development. The Productivity dimension consists of three categories:

Feeling productive
Maybe feeling productive
Feeling nonproductive

Manager Role Dimension

There are situations when a manager operates as a specialist, rather than a manager. This is the situation if a corporate treasurer executes simple bookkeeping tasks, or if the president chooses to teach a class in a discipline that he masters. A newly promoted manager may frequently return to his specialist role, in which he feels more secure than in his managerial role. Thus, measuring the amount of time in which a manager assumes a specialist role indicates his adaptation to his position. The Manager Role dimension consists of the following:

Acting as Manager
Acting as Specialist

There are, of course, occasions when such a distinction is difficult to make. Particularly when specialist functions overlap with categories in the management role dimension is there room for discussion as to what would be the appropriate coding procedures.

Stress Dimension

The amount of stress which a person feels may be related to his productivity, his job satisfaction, or other aspects of his position. Therefore, a measure of stress was included, as follows:

Stressed
Relaxed

Data measurement began on February 13, 1974, with the installation of the units. The study was run for six weeks, the first week being a test of the adequacy of the code list. Because several participants worked during their lunch hour, the units were on from the moment participants reached the office until they left for home. In addition, if they did work away from the office, they were to enter any pertinent information upon returning to their offices, estimating four signals per hour. This stipulation included work at home, particularly over weekends. In this way, a more realistic picture of each person's job was obtained.

A contact person, who is the liaison between Extensor and project participants, was chosen. He was a member of the management group, and a participant in the study. It was his responsibility to answer questions posed by study members and refer anything which he could not explain to Extensor. In addition, he passed on comments to participants from Extensor concerning any problems with punching or other areas which was noticed after reviewing the test measurement results.

FINDINGS

Time Illusion

Prior to the initiation of the Study, each participant estimated the distribution of his time for his instrumental activities and his functional activities. To test the extent of the "time illusion" among the participants, the estimate versus actual distribution for each participant was tested by χ^2 for significant differences. Only 2 of the 7 participants had distributions where the null hypothesis of no difference could not be rejected (and even for these, there were marked differences between the estimated versus actual distributions —but not strong enough to completely rule out chance variations) at $p < .005$. For the functional estimates, only 1 of the 7 participants came close enough to avoid rejection. There is thus clear evidence that the participants did not have a correct picture of how their time was being spent. This is an important finding, because a large amount of planning and decision making is executed on the basis of data submitted in questionnaire form, using individual estimates of the type used above for the time estimates. It was also an interesting learning experience for each participant to note where his subjective estimates differed from his actual findings. The findings of the study are presented below.

Instrumental Activities

The single most important finding is the large amount of time spent in meetings, 66.3%. This corresponds to an average of 26.5 hours per week, considerably more than the 22 hours (55%) that was estimated in advance.

TABLE I

Average and Range of Time Spent on Instrumental Activities by the Management Group

	Averages		Range	
	Preestimate	Actual	High	Low
1. Talk, meet 1:1	27.9%	28.0%	34.8%	20.0%
2. Talk, meet 4 or less	12.1%	12.0%	17.2%	4.8%
3. Talk, meet 5 or more	15.0%	26.3%	43.3%	13.7%
4. Telephone IN	6.0%	2.7%	3.8%	1.3%
5. Telephone OUT	4.7%	3.3%	4.7%	1.2%
6. Write, dictate, punch	13.1%	13.0%	23.3%	4.5%
7. Think	3.0%	.8%	1.6%	0
8. Read	5.1%	4.1%	11.8%	1.5%
9. Study	6.1%	4.3%	10.7%	.7%
10. Edit, Sign	3.6%	1.2%	2.2%	.1%
11. Sort, file, retrieve	1.4%	1.8%	6.8%	.2%
12. Other	2.0%	2.5%	12.8%	0
	100.0%	100.0%		

Individual variations are also large for all types of meetings, they range from 43.3% to 13.7% for 5 or more (average 26.3%), from 34.8% to 20.0% for face-to-face encounters (average 28.0%), and from 17.2% to 4.8% for 2 to 4 participant meetings (average 12.0%). Only 6% of the time is consumed by telephone calls, whether externally or internally initiated. Considerable variation is found for Write, dictate, punch: 23.3% to 4.5%, with an average of 13.0%. This was almost exactly what was estimated in advance. Time to think as an unrelated instrumental activity was estimated in advance at 3.0%, or 80 minutes per week—in reality it emerged at .8%, or about 19 minutes per week.

Time to read ranged from 11.8% to 1.5%, with an average of 4.1%—slightly underestimated.

Time to study ranged from 10.7% to .7%, with an average 4.3%—somewhat below the 6.1% estimate.

Edit, sign only consumed 1.6% of the time compared with a pre-estimate of 3.6%.

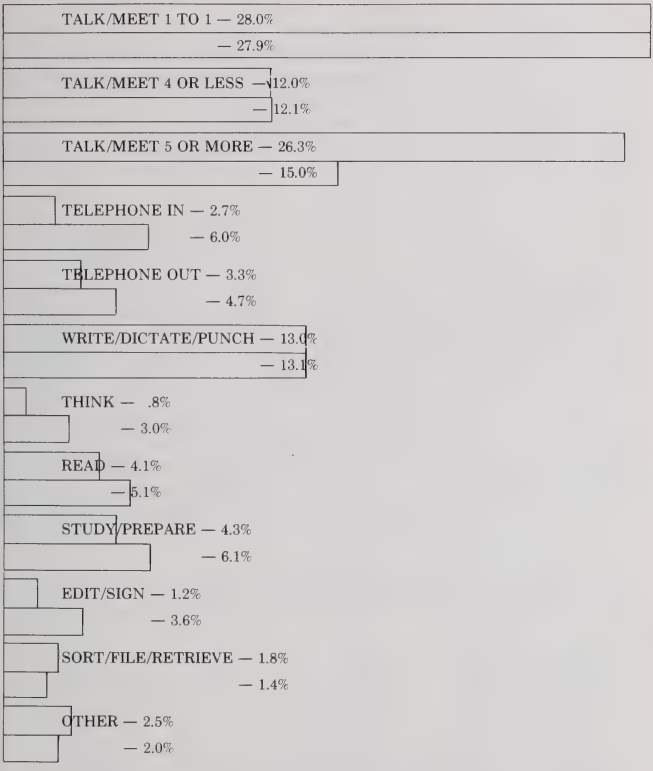
The manual routines of sort, file, retrieve ranged from 6.8% to .2%, with an average of 1.8%. This is an activity which should be virtually eliminated through a more effective use of secretaries, file clerks or assistant personnel—if this is done, thinking time, for example, could be tripled.

From a pragmatic viewpoint, the key category is the meeting time category. The most important reallocations of time are likely to take place within the three meeting categories.

Contact Dimension

Since each participant had a different code for interactions, it is not possible to show a complete contact dimension without violating confidentiality. The contact dimensions represented a socio-grammatic picture of the internal communication patterns of the group, and identified the channels and

ACTIVITY DIMENSION
GROUP AVERAGE (Upper bar)
ESTIMATES AVERAGE



pathways of their interactions for each individual. This is a most interesting dimension from the participant's point of view, and it uncovered "islands of non-communication" that spurred a number of changes in communication patterns subsequent to the presentation of results.

Initiative Dimension

The management group tends to initiate its own activities to a large extent. As expected, a cross tabulation of the data confirms that initiative is correlated with rank: The higher up in the organization, the larger the percentage of actions taken on the executive's own initiative.

Planning Dimension

Predominantly the use of time is planned in this organization, but variations in planned time is not strongly correlated with rank. It is surprising that so much of the work is planned, given the supposed erratic changes in health care seeking behavior of the public. The findings underscore the results of other investigators indicating that predictability of hospital demand, and therefore also hospital functioning, may be high if the cyclicities of demand are known to hospital officials.

Delegation Dimension

This is a crucial dimension for judging appropriateness of work activities. It is remarkable that 88.5% of the work is not judged to be delegatable by the participants. This may indicate that the work is already optimally divided, or that skills in delegation need to be taught to the group. In any case, 6.1% to 11.5% of the work could be delegated by the participants' own judgment—

TABLE II

Average and Range of Time Spent on Activities Initiated by the Management Group

	Average	Range	
		High	Low
Own Initiative	64.8%	75.2%	53.0%
Other Initiative	35.2%	47.0%	24.8%
	100.0%		

TABLE III

Average and Range of Time Spent on Planned Activities by the Management Group

	Average	Range	
		High	Low
Planned	80.3%	92.7%	67.4%
Unplanned	19.7%	32.6%	7.3%
	100.0%		

TABLE IV

Average and Range of Time Spent on Activities Subject to Delegation Judgments by the Management Group

	Average	Range	
		High	Low
Delegate Now	6.1%	13.2%	1.0%
Delegate Maybe	5.4%	20.3%	.6%
Do not Delegate	88.5%	97.2%	66.5%
	100.0%		

freeing anywhere from 120 to 230 hours per year for other purposes for each participant.

Functional Dimension

This dimension constitutes the inventory of job activities for each individual participant, and consequently is different for each participant. Thus no data can be provided without violating confidentiality.

It should be mentioned that the value of a completely updated and accurate job description is considerable. The efficacy of management by objectives can be judged on the basis of what each objective consumes of resources, leading to a reassessment of appropriate objective generation. The results may also be used in personnel management to train successors to take over jobs where the functions have been clearly defined and measured.

Management Role Dimension

Slightly more than one-fifth of the time is spent on planning. Planning was defined as "determining goals, policies and courses of action; work scheduling budgeting, setting up procedures, preparing agendas, programming." Since 80.3% of all events are planned, there is a ratio of approximately 1:4 between planning, and the amount of time in total that is affected by the planning process.

The category of investigating, which encompasses "collecting and preparing financial statements, record keeping, performing research, job analysis," consumes 18.3% of the time, with a range from 42.0% to 6.1%. Coordinating is mainly a process of working with and through other departments—this takes 16.8% of the participants' time, with a range from 26.7% to 9.6%.

Evaluating, which emphasizes the exercise of judgment by employees, and

TABLE V

Average and Range of Time Spent on Management Role Activity by the Management Group

	Average	Range	
		High	Low
1. Planning	21.3%	31.2%	8.8%
2. Investigating	18.3%	42.0%	6.1%
3. Coordinating	16.8%	26.7%	9.6%
4. Evaluating	10.1%	20.7%	1.6%
5. Supervising	8.0%	20.3%	.7%
6. Staffing	2.7%	5.0%	.8%
7. Negotiating	3.7%	15.0%	.4%
8. Representing	9.7%	20.6%	3.9%
9. Educating	3.2%	8.8%	.4%
10. Developing Constituencies	1.8%	3.9%	0
11. Personal	1.5%	4.0%	.1%
12. Other	2.9%	7.6%	0
	100.0%		

representing, which involves community and health agency relations, consume almost equal amounts of time, 9.9% (with a range of 20.7% to 1.6%) and 9.7% (with a range of 20.6% to 3.9%). Supervising, which involves directing, counseling and training subordinates, consumes 8.0% with a range of 20.3% to .7%.

The other categories consume much less time than the above: Staffing (2.7%), negotiating (3.7%), educating 3.2%), developing constituenceis (1.8%) and personal (1.5%).

Productivity Dimension

Although there are wide variations in the "sense of productivity" reported (91.8% to 56.6%), the average of 76.5% indicates a high level of productivity perceived by the participants. However, this is a valuable classification when it comes to the evaluation of the data; each individual can match his nonproductivity classification against the relevant activities or functions in two-way tabular formats.

Manager Role Dimension

As expected, this classification was strongly correlated with rank—the higher up in the organization, the more time is spent as a manager versus as a specialist.

Stress Dimension

Although the average amount of stress felt in the organization is only 7.6% of the time, the large range (25.5% to .5%) warrants a closer examination. This is

TABLE VI

Average and Range of Time Spent on Activities Grouped by Sense of Productivity by the Management Group

	Average	Range	
		High	Low
Feeling Productive	76.5%	91.8%	56.6%
Maybe Feeling Productive	20.5%	40.4%	6.8%
Feeling Nonproductive	3.0%	6.8%	1.4%
	100.0%		

TABLE VII

Average and Range of Time Spent on Activities Grouped by Manager-Specialist Categories by the Management Group

	Average	Range	
		High	Low
Manager	73.7%	98.9%	29.5%
Specialist	26.3%	70.5%	1.1%
	100.0%		

MANAGEMENT ROLE DIMENSION
GROUP AVERAGE

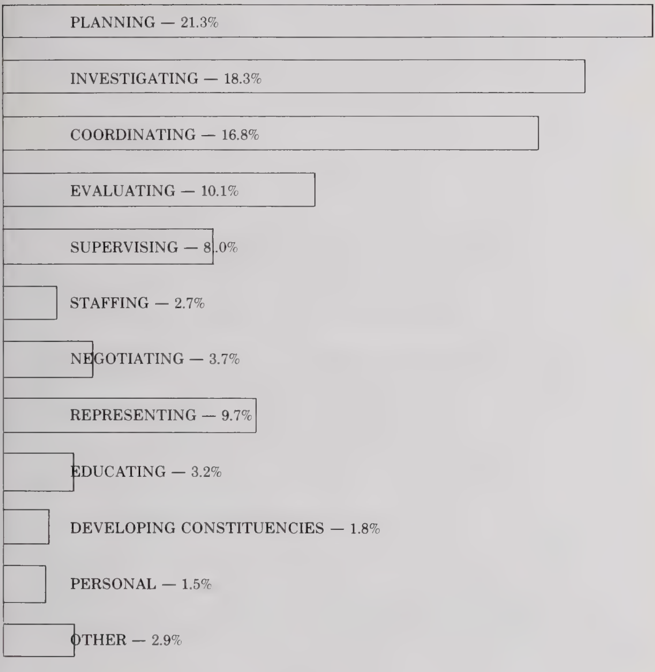


TABLE VIII

Average and Range of Time Spent on Activities Grouped by Stress Categories by the Management Group

	Average	Range	
		High	Low
Stressed	7.6%	25.5%	.5%
Relaxed	92.4%	99.5%	74.5%
	100.0%		

done in another paper (9). The sources of stress were identified and quantified, and presented to the participants during the evaluation process.

The 10 dimensions presented in this paper, may be analyzed in 1 × 1 tables. This will produce a total of 45 two-way tables, where each dimension may be cross-classified against every other dimension. It will be prohibitively space consuming to repeat these tables here, although they were all prepared for the management group. Suffice it to say, that each individual participant used all relevant cross-tabulations to track down his personal sources of delegable work, stress, nonproductivity, unplanned work and specialist behavior. With the insights gained from this process, the evaluation phase could be initiated.

EVALUATION OF FINDINGS

The Concept of a Time Budget

The evaluation process consists of three separate steps:

1. Each participant reviews his personal reports and determines how much time can be *released* by identifying time waster activities, and by taking appropriate steps to correct these.
2. Each participant develops his own list of priority items that he wants to address himself to on the job. The steps needed to realize these priorities are enumerated, and the time *required* is identified.
3. Each participant now formulates his Time Budget. On the left hand side, the time-waster activities, uncovered by the participant, are identified. Next to these, the action steps needed to eliminate these activities are listed. Finally, the amount of time saved, e.g., in hours per week, is listed in the final column of the left hand side of the Time Budget.

On the right hand side, the individual participant lists the priority items that will replace the time waster activities identified through the analysis. The action steps needed to realize these priority items are listed next; finally the amount of time needed to complete these steps is listed in the final column on the right hand side of the Time Budget (Appendix B).

This is a description of the conceptual process behind Time Budget formulation. In reality, a series of practical steps is needed to arrive at an operational Time Budget.

Practical Procedures

In order to complete the time budget, the following sequence is usually needed:

1. The participants must be introduced to the field of time management. When a summary of the state of the art of time management is given, the participants acquires a sense of analytical direction in analyzing their own data. This is most helpful in preparing the left hand side of the Time Budget.
2. There is no purpose in releasing time for other activities if these activities have not been identified and prioritized. Consequently, the participants are put through a rather demanding process where their goals and objectives on the job are identi-

fied, prioritized and eventually planned for execution. The procedure used by Alan Lakein (5) may be used to operationalize this process.

3. Once the time budget has been formulated, a plan for implementing the action steps, complete with deadlines, must be worked out by each participant. The items that require changes affecting other members in the organization must be submitted to the management group for approval. When management has endorsed these items, the commitment of each individual becomes the driving force in the implementation phase.

Outcome

Through a series of meetings, the process of developing the Time Budget was completed. A large number of suggestions were generated by the group, indicating strong commitment and interest in the project. Since each individual Time Budget is a confidential document, a sample Time Budget has been appended as Appendix B. It represents a composite of items from several individual Time Budgets.

There seems to be little doubt that most managers should be able to clear about 25% of their time with little or no drop in current output. The idea for this management group, who worked an average of 55 hours a week, was to approach a normal work week. Time represents the ultimate energy crisis as a resource. It can neither be expanded nor contracted, and its effective management is a precondition for any other form of effective management.

Summary and Conclusions

Time Management as a Process

Until recently, there was no economic, accurate or effective system randomly to sample behavior, attitude and judgment in an organization. And unless such a data base can be produced, the presence of a substantial time illusion would hamper the accurate assessment of proper time management procedures.

The Extensor system supplies a rich, complex and unusually precise data base that permits an in-depth analysis of organizational functioning, individual interaction and attitudinal sets. The data in this study revealed an organization that engaged in frequent meeting behavior between individuals that were used to act on their own initiative, in a planned manner, oriented to tasks that they generally did not feel could be delegated. They operated as managers more than specialists, emphasizing the functions of planning, investigating, coordinating, evaluating and supervising. They generally felt productive and relaxed. Upon being presented with the results from the study, they identified a series of action steps that should be initiated in order to improve their present time allocations. These improvements were formalized in individual Time Budget form, developed by the participants themselves.

Clearly the employee-initiated changes in behavior are easier to institutionalize than changes ordered from above by fiat. In addition, the fact that the participants are studied *on the job*, makes it possible to examine organizational problems and concerns where and when they exist. This is a rather different approach to manpower development from that of sending employees to outside training programs. In the latter case, assuming that the program topics are appropriate, the organization loses the seminar participants' time away from work, and is faced with a probable conflict upon the time of their return. The reason for this is the fact that the organization is unlikely to have changed its ways in the interim, while the seminar participants quite likely have undergone changes in both attitude and judgment. There is thus the potential for conflicts between the inertia of organizational change and the frustration of individual change agents. On-the-job manpower development seems to stand a better chance of dealing with changes, since they can be addressed by everyone within a shared work environment.

In this study, time management was the theme of the overall manpower development project. The project had to start by the participants establishing a precise baseline data base for all dimensions of interest to them. Then the skills for approaching and analyzing the data had to be imparted in order to identify the desirable changes in individual behavior. Finally, the changes had to be institutionalized through a process of communication, commitment and motivation.

The institution had identified several overriding objectives that the study addressed directly:

1. It was important to demonstrate that the management group was not excluded from the kind of in-depth study and analysis that had recently been applied to nonsupervisory personnel in the hospital.
2. It was desirable to reduce the oppressive sense of time pressure that affected all participants of the management group. The average work week fluctuated between 50 and 55 hours, necessitating frequent evening and weekend work.
3. It was necessary to develop a data base that could serve as a basis for a management development effort to improve communication and effectiveness of each member of the group, as well as the group itself.

At the time of this writing, 3 months after the completion of the study, it is now clear that the institutional objectives have been accomplished:

1. Nonsupervisory personnel are aware of and satisfied that the management group has kept faith with them by participating in the same kind of rigorous self and consultant analysis as did nonsupervisory personnel.
2. The average work week for the management group has been decreased by approximately 5 hours per week.
3. Attitudes and work schedules for all individuals, as well as for

the management group have been significantly modified as a result of the study.

A more detailed and objective analysis will be needed to determine the eventual outcome of the effect of the study and its implementation. Both the institution and Extensor contemplate a follow-up study within a year of the completion of the original study.

The Potential for Further Research

The development of a large and precise data base over a period of 6 weeks at little expense has major implications for organizational analysis. It is now possible to collect data on a vast variety of problems previously thought to be impossible to examine. Queuing studies can be done with great precision and with little expense. Studies on communication patterns, job satisfaction, executive stress and work behavior may be performed and the results fed back in less than two months. The implications for decision making and manpower development are quite impressive.

If scalar dimensions are used, the individual's delegation dimension can be expressed as a function of all other variables. If a linear regression form is used, needed delegation can be expressed as a product of the intensity that the individual feels towards delegating a particular task (the regression coefficient) and the duration of the task (the percentage of time consumed by the task). Thus an individual's activities can be ranked by their delegability, indicating to the individual where an appropriate intervention and delegation should take place.

What can be done for an individual, can be done for an organization by summing across individuals for common discussions. Thus the delegation potential for an organization can be identified and studied in detail.

Similarly stress, job satisfaction and initiative can be studied, both on the individual and the organizational levels. In fact, it is possible to simulate changes in stress likely to be associated with changes in certain activities or behaviors. It thus becomes possible to experiment on equations, rather than humans—an effective and proven procedure of long standing in fields other than management. This should open the way for testing and evaluating theories of management and human behavior that previously had proven difficult or impossible to do. Already studies of this kind are under way in a number of settings across the United States. Eventually it is hoped that patterns of effective, executive behavior can be identified and analyzed in the context of both for-profit and nonprofit environments.

Acknowledgments

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Appendix A

Definitions of Management Role Categories

PLANNING	Determining goals, policies, and courses of action; work scheduling, budgeting, setting up procedures, preparing agencies, programming.
INVESTIGATING	Collecting and preparing information, usually in the form of records, reports, and accounts; inventorying, measuring output, preparing financial statements, record keeping, performing research, job analysis.
COORDINATING	Exchanging information with people, other than subordinates, in the organization in order to relate and adjust programs; advising other departments expediting, liaison with other managers, arranging meetings, informing superiors, seeking other departments' cooperation.
EVALUATING	Assessment and appraisal of proposals or of reported or observed performance; employee appraisals, judging financial reports, approving requests, judging proposals and suggestions, reviewing quality of care.
SUPERVISING	Directing, leading, and developing subordinates; counseling and training subordinates, explaining work rules, assigning work, disciplining, handling complaints of subordinates.
STAFFING	Maintaining the work force; employment interviewing, selecting placing, promoting and transferring employees.
NEGOTIATING	Purchasing or contracting for goods or services; contracting and dealing with suppliers, collective bargaining.
REPRESENTING	Advancing general organizational interests through memberships, speeches, consultation, and contracts with individuals or groups outside the organization; public speeches, community drives, news releases, attending conventions.
EDUCATING	Active participation in teaching.
DEVELOPING A CONSTITUENCY	Obtaining the support of individuals and groups, inside and outside the organization; doing favors, selling ideas.

Appendix B

Time Budget

Time waster	Action Steps	Time available	Priority item	Action steps	Time needed
1. Meetings	1. Reduce all to 1 hour. 2. Prepare + distribute agenda to all participants at least 24 hours before meeting. 3. Set priorities on items on agenda. 4. Start meetings on time; devote most time to items of highest priority. 5. Determine whether meetings are necessary. 6. Delegate meetings, especially 1:1, to relevant assistants.	3:0	1. Operate hospital on sound fiscal basis	Review finances once a week.	1.0
			2. Develop prepaid program	1. Develop plan. 2. Identify decision process. 3. Pursue preferred options. 4. Implement plan.	2.5 (ave.)
			3. Expand knowledge of health care delivery	Read journals, reports and other informational materials.	1.25
					4.75 hours per week
2. Mail, screening, routine	Delegate to secretary	.5			
3. Newspaper + periodical screening	Delegate to secretary	.5			
4. Minute Preparation	Delegate to secretary	.75			
		4.75 hours per week			

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The Effects of Immunoglobulins and Nonenzymatic Proteins on Leucocyte Migration*

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Abstract

In 54 of 100 experiments 0.1 mg protein from antisera raised in goats to human immunoglobulin (Ig) chains and IgG fragments induced 25% or more leucocyte migration inhibition (LMI) while the same quantity of protein from the Ig chains themselves induced 25% or more LMI in 2/3 of the experiments. LMI by 0.1 mg protein from antisera to albumin and transferrin and from normal goat serum induced less than 25% LMI in 20 of 21 experiments. Complement inactivation always increased LMI by protein from antisera or Ig chains.

The effect of different concentrations of nonenzymatic protein markers (Cytochrome C, Chymotrypsinogen A, Ovalbumin, Bovine Albumin, Apoferritin) on LMI was investigated. 0.1 mg protein/ml did not cause LMI. Heat denaturation (56°C for 30 min) induced 25% or more LMI in one-third of experiments. When the protein concentration was increased to 1 mg/ml 25% or more LMI was produced in one-fourth of experiments.

It appears that LMI can be produced by aggregates of protein unrelated to cell membrane determinants, perhaps by hindering cell migration. Antigen-antibody complexes may bind to membrane determinants of all types of leucocytes, thus leading to their aggregation and migration inhibition.

The mediators of cellular immunity or lymphokines, are secreted by T lymphocytes which bear few monomeric μ and light chain determinants (1–3). Sensitized human lymphocytes, after incubation with antigen, produce migration inhibitory factor(s) MIF which inhibit guinea pig macrophage migration (4). In the direct in vitro test, the migration of leucocytes is inhibited by the simultaneous addition of antigen. A greater quantity of antigen is required but there is no need for incubation and the results are available within 24 hours (4, 5).

Human B lymphocytes are recognized by their immunoglobulin (Ig) and complement membrane determinants (6). The effect of antisera raised in goats to human Ig chains of all classes and to IgG fragments (IgG Fab and IgG Fc) was checked in the direct leucocyte migration inhibition (LMI) test. Antisera to human albumin and transferrin, normal goat serum, and nonenzymatic protein markers of differing molecular weights were used as control proteins. Experiments were performed with Ig and antisera before and after complement inactivation and with the control proteins after heat denaturation (h.d.). The white cells of volunteers were used whose sensitivity to Ig, antisera, and nonspecific proteins was not checked by in vivo skin tests or other in vitro tests.

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Materials and Methods

The subjects were 11 healthy volunteers from The Mount Sinai Hospital and laboratory personnel. They had normal serum titers of γ, α, and μ chains determined by immunodiffusion (7) (Meloy Laboratories, Springfield, Virginia). Their leucocytes were used on three to five occasions to assess the response to all the antigens. No individual had received a transfusion or gammaglobulin injection within the last two years. A total and differential white blood cell count was performed with each bleeding.

Antigens: Immunoglobulin chains, γ, μ, κ and λ and antisera raised in goats to human Ig chains (γ, α, μ, ϵ, κ, and λ) albumin and transferrin were obtained from Meloy Laboratories, (Springfield, Virginia). Goat antiserum to human IgG Fab and IgG Fc was obtained from Miles Laboratories, (Kankakee, Illinois). Experiments were performed with normal goat serum (NGS) obtained from both laboratories. The Ig chains, antisera and NGS were tested at a protein concentration of 0.1 mg/ml.

Proteins of known molecular weight, Cytochrome C, M.W. 12,400, Chymotrypsinogen A, M.W. 25,000, Ovalbumin, M.W. 45,000, Bovine Albumin, M.W. 67,000, Apoferritin, M.W. 480,000 were obtained from Schwarz/Mann, (Orangeburg, New York) and used in different concentrations.

The leucocyte migration inhibition test has been previously described (7, 8). Twenty-five percent or more LMI is considered significant in this laboratory.

Experimental Procedures

The volunteers' leucocytes were tested for LMI by adding 0.1 mg protein from each of the antisera, NGS and Ig chains listed above to the 1.0 ml of medium contained in the migration chamber (Fig. 1). LMI induced by 0.1 mg protein from γ, μ, κ, and λ chains was compared to LMI induced by antisera to

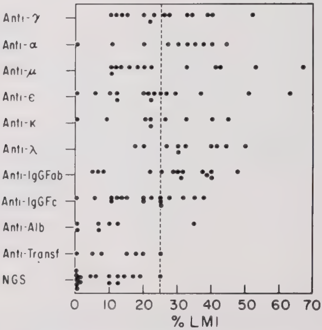

FIG. 1. LMI by 0.1 mg/ml protein from antisera to Ig chains and fragments, Albumin (Alb), Transferrin (Transf), and Normal Goat Serum (NGS).

these chains (Fig. 2). The effect of the same proteins on LMI after complement inactivation was tested (Fig. 3). LMI was studied with nonenzymatic protein of different molecular weights in concentrations ranging from 0.1 to 1.0 mg/ml, and with 0.1 mg protein heated for 30 min at 56°C (Fig. 4).

The purity of the antisera had been ascertained earlier (7). Albumin and γ chain were mixed with their respective antisera which led to precipitate formation after incubation for one hour at room temperature. The supernate was tested for LMI after separation from the precipitate by centrifugation at 20,000 × G. The chambers were opened after the experiments and the cells in the migration fan on the inferior cover glass were stained with trypan blue.

Results

Antiserum protein 0.1 mg against the various Ig chains induced 25% or more LMI in 54 of 100 experiments (Fig. 1). Anti-albumin induced this extent of LMI in 1 of 7 experiments while neither anti-transferrin or NGS induced 25%

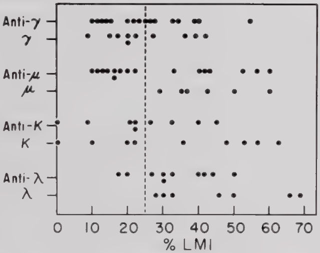

FIG. 2. LMI by 0.1 mg/ml protein from Ig chains and antisera to Ig chains.

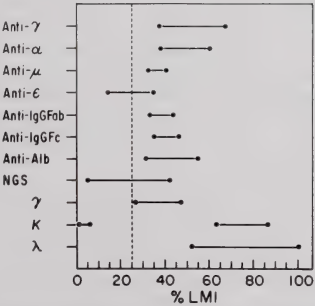

FIG. 3. Horizontal lines link LMI induced by 0.1 mg/ml protein from antisera, NGS and Ig chains prior to complement inactivation to the increased value thereafter (Fig. 2 re abbreviations).

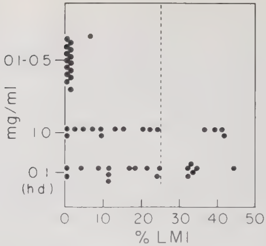

FIG. 4. Similar LMI results were obtained with the nonenzymatic protein markers Cytochrome C, M.W. 12,400, Chymotrypsinogen A, M.W. 25,000, Ovalbumin, M.W. 45,000, Bovine Albumin, M.W. 67,000 and Apoferritin, M.W. 480,000. The results have been combined at concentrations of 0.1–0.5 mg/ml, 1.0 mg/ml, and 0.1 mg/ml heat denatured (h.d.).

LMI in 14 experiments. Statistical comparison between experiments carried out with antisera against Ig chains and with other antisera or NGS indicate x^2:$p < 0.01$. The results with NGS obtained from the two laboratories were similar and are combined in Fig. 1. Protein from Ig chains induced LMI in ⅔ of experiments (Fig. 2). Complement inactivation (56°C, 30 min) always increased LMI (Fig. 3).

Nonenzymatic protein markers were tested for LMI at several concentrations between 0.1 mg/ml and 1.0 mg/ml. LMI by Cytochrome C, Chymotrypsinogen, Ovalbumin, Bovine Albumin and Apoferritin, ranging in M.W. from 12,400 to 480,000 was in the same range and all results are combined (Fig. 4). There was no LMI at concentrations below 0.5 mg/ml. 1.0 mg of these proteins caused more than 25% LMI in ¼ of experiments and 0.1 mg heat denatured protein (h.d.) produced LMI in ⅓ of the experiments (Fig. 4).

The supernates, separated from the precipitates of γ chain or albumin with their respective antisera, induced no LMI. Antisera and Ig chain protein induced LMI to different extents in the various volunteers (Fig. 1) which could not be correlated with the total or differential white blood cell count. It had been shown earlier that LMI by anti-Ig was not related to the individual's serum Ig level (7). Repeated testing with the same antigen in some individuals resulted in differences of 10% or less. More than 90% of cells demonstrated viability at the conclusion of the experiments by trypan blue exclusion.

Discussion

Protein 0.1 mg/ml from antisera to human heavy and light chains, induced leucocyte migration inhibition (LMI) in 54 of 100 experiments while 0.2 mg/ml almost always induced 25% or more LMI (7). Since only light chain and monomeric μ chain determinants have been reported on T lymphocytes it appears unlikely that LMI induced by antisera to all Ig chains is mediated by T cells (2, 5). B lymphocytes have abundant Ig determinants and are between one-half and one-third as frequent as T cells in the circulation of healthy individuals; they can secrete MIF (6, 9, 10). LMI by anti-α has been reported in two patients with IgA deficiency (7), in whom B cells but not T cells with α membrane determinants may be present (11).

The finding that anti-ϵ induces LMI as frequently as anti-μ supports the concept that the number of Ig membrane determinants are not related to humoral Ig levels in the circulation (7, 12). Of equal interest is the case of the volunteer with detectable κ chain by Ouchterlony immunodiffusion who showed no LMI by 0.1 mg protein from κ chain or antiserum to it and barely enhanced LMI after complement inactivation (7) (Figs. 1–3). The same quantity of protein from anti-IgG Fab induced 25% LMI more often than anti-IgG Fc. No explanation can be offered for consistent LMI by μ and λ chains, but not by κ chains, all of which are T cell membrane determinants. Ig chains induced 25% LMI more frequently than antisera to them. Anti-IgG Fab and Ig chains appear to react with more membrane determinants than antisera to IgG Fc and antisera to Ig chains respectively, the latter reacting with the Ig class Fc fragment (Figs. 1, 2).

LMI may be due to the aggregation of Ig molecules at cell membrane sites with linkage to other cells resulting in cellular aggregates hindering cell migration. Leucocytes other than lymphocytes may be involved since receptors for IgG have been described on human monocytes & PMN's (13, 14). The mechanism is comparable to antigen-antibody-complement complexes inhibiting macrophage migration in guinea pigs (15). The migration of PMN's is also influenced by their phagocytosis of aggregate IgG or immune complexes with the release of lysosomal enzymes which affect leukotaxis (9, 16). Recently PMN migration inhibition has also been attributed to the release of leucocyte migration inhibitory factor (LIF) by sensitized B and T cells (2).

Anti-albumin appears to react with fewer white cells since albumin is a rare membrane determinant (17). Concentrations of 0.2 mg protein from anti-albumin and from NGS induced more than 25% LMI frequently (7). These observations suggest a relationship between the number of leucocytes bearing determinants and the quantity of antigen used in the LMI test (4, 5). No LMI was induced by anti-transferrin, NGS and nonenzymatic proteins at a concentration of 0.1 mg/ml. At a concentration of 1.0 mg/ml, the nonenzymatic protein caused more than 25% LMI in 25% of the experiments and they did so after heat denaturation at 0.1 mg/ml concentration in 33% of experiments. Vital staining indicated that the proteins were nontoxic to the leucocytes. Protein molecules in larger quantities or aggregated molecules unrelated to membrane determinants may, therefore, also be an obstacle to leucocyte migration.

Human leucocyte migration inhibition (LMI) has been studied as an in vitro test for cell-mediated immunity in numerous disorders (8, 18–22). Particulate and soluble tissue extracts as well as cellular organelles have been used as antigen. LMI correlates with disease activity since the antigens do not produce LMI in healthy individuals. LMI may be caused by antigen interacting with membrane determinants not yet discovered on T lymphocytes or the Fab domain of monomeric IgM and light chains, on or within T cell membranes (12, 23) or with a histocompatibility-linked immune gene response product (24). The antigen may react with this and other domains or Ig membrane determinants of B cells as well as with Ig molecules of other leucocytes (2, 6, 13, 14, 20). The patient's response to antigen in the LMI test is enhanced by prior sensitization, the antigen dose, and the number of sensitized leucocytes (5). LMI can also result when antigen determinants are present and there has been no prior sensitization. Until leucocyte migration and its inhibition are understood, it will be difficult to attribute an exact immunologic significance to the results of LMI (25, 26). The present study suggests that antigen reacting with leucocyte membrane determinants may cause migration inhibition, and that protein aggregates unrelated to membrane determinants can impede migration.

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Cyclic GMP and Leucocyte Migration Inhibition Induced by Antiserum to Human Immunoglobulin and by Dexamethasone*

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Abstract

Leucocyte migration inhibition (LMI) has been widely used as an in vitro test for cell-mediated immunity. It can be induced by protein from antisera to the various human immunoglobulin chains in healthy individuals not known to be sensitive to the proteins. Twenty-five percent or more LMI can also be induced 0.6×10^{-4}M and larger doses of dexamethasone. The addition of 1 nM cyclic GMP did not influence LMI induced by anti-Ig, whereas steroid-induced LMI was reduced in all but four of 32 experiments. Neither cyclic GMP or cyclic AMP produced LMI and cyclic AMP did not influence LMI induced by DMS. The implications of these findings are discussed.

Leucocyte migration inhibition has been used as an in vitro test by laboratories to assess cell-mediated immunity (1–6). Soluble proteins from homogenates of various tissues of homologous eyes have been found to induce leucocyte migration inhibition (LMI) in patients with ocular disorders (2, 4). Leucocyte migration is inhibited by 0.6×10^{-4}M dexamethasone to a greater extent in a greater percentage of patients with open angle glaucoma and diabetes mellitus than in healthy volunteers (7, 8).

Immunoglobulin (Ig)-bearing leucocytes may be involved in the LMI test whereas only T lymphocytes are considered mediators of cellular immunity (9-13). Antigen recognition by T cells is not understood. Relatively few monomeric μ and light chain immunoglobulin (Ig) molecules have been detected on the surface of T cells, which constitute approximately 75% of circulating lymphocytes (12, 14); B lymphocytes represent 25% of the circulating lymphocyte pool but carry numerous Ig membrane determinants of all classes (12, 15). Antisera to IgG fragments and to Ig chains as well as the Ig chains themselves induced LMI in healthy volunteers who were not tested for cellular sensitivity by other in vitro or in vivo tests (10, 11). 0.2 mg protein from antisera to all classes of heavy and light chains induced 25% or more LMI while 0.1 mg protein induced 25% LMI in 54% of experiments with the leucocytes of these volunteers. All Ig-bearing cells including monocytes and polymorphonuclear leucocytes (PMN's) may play a role in LMI induced by anti-Ig with the initial antigen-antibody reaction taking place at the cell membrane (9–11, 13, 16).

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Steroids also react with the cell membrane and subsequently with cytoplasmic organelles and the nucleus (17, 18). Experiments were carried out to study the effect of cyclic GMP and cyclic AMP, which play a role in intracellular metabolism on LMI induced by anti-Ig and by steroids (7, 18–21).

Materials and Methods

Leucocytes were obtained from healthy volunteers of The Mount Sinai Hospital personnel and from patients from the Ophthalmology Out-Patient Department who were free of disease. Many were tested repeatedly and white blood cell counts and differentials were obtained with each venopuncture.

Normal goat serum (NGS) and antisera raised in goats to human, γ, μ, ϵ, κ, and λ chains were obtained from Meloy Laboratories (Springfield, Va.); Dexamethasone Sodium Phosphate Ophthalmic Solution, 0.1% (DMS) with sodium bisulfate 0.32%, phenylethanol 0.25% and benzalkonium chloride 0.2% and the ophthalmic solution without DMS were provided by Merck, Sharp & Dohme (West Point, Pa.). Cyclic GMP and cyclic AMP, 3′:5′ cyclic monophosphoric acid, sodium salts, were purchased from Sigma Chemical Co. (St. Louis, Mo.).

The leucocyte migration inhibition test has been described in earlier publications (2, 10).

Experimental Procedures

Leucocyte Migration Inhibition (LMI) was studied with 1 mg protein from antisera to various human Ig chains, added to the 1 ml of medium in migration chambers containing leucocytes from volunteers. In simultaneous experiments various concentrations of cyclic GMP were added to the antiserum protein. The migration of leucocytes from the same volunteers were also tested with:

1. cyclic GMP in concentrations ranging from 10^{-10} to 10^{-4}M/ml
2. dexamethasone 0.6×10^{-4}M/ml, 0.3 and 1.2×10^{-4}M/ml with and without the above concentrations of cyclic GMP.
3. cyclic AMP in concentrations from 10^{-9} to 10^{-4}M/ml
4. dexamethasone 0.6×10^{-4}M/ml with and without concentrations of cyclic AMP ranging from 10^{-9}M to 10^{-5}M/ml and with the ophthalmic suspension without DMS.

The migration fans were magnified by projection, traced, cut out, and the cut-outs weighed as previously described in detail (2, 10). Cell viability was assessed with trypan blue at the end of the experiments.

Leucocytes from patients who had been seen in the ophthalmologic outpatient department were tested with dexamethasone and cyclic GMP as in (2) above. Volunteers were tested repeatedly to assess reproducibility of results.

Results

LMI to variable extent was induced with the cells of each volunteer by either 1 mg protein from antisera to Ig chains or 0.6×10^{-4}M DMS (30 μg dexamethasone phosphate) (Fig. 1). The addition of cGMP reduced LMI induced by DMS significantly ($t_{25} = 5.25$, $p < .001$) whereas it did not affect anti-Ig induced LMI ($t_{16} = 0.1$, $p > .90$). When the same volunteers were tested with both DMS and anti-Ig the influence of cGMP was significantly greater in the presence of DMS, ie 8.89% vs 1.04% respectively $t_8 = 2.36$, $.02 < p < .05$ (8 d.f.). There were no differences in cGMP influence on DMS-induced LMI between the volunteers and the patients from the ophthalmology outpatient department (Fig. 1).

LMI was increased by doubling and decreased by halving the quantity of DMS (not shown). Other concentrations of cGMP, and no concentration of cAMP affected the migration pattern induced by anti-Ig or DMS even when the protein concentration was reduced to 0.2 mg/ml or the concentration of DMS was reduced to 15 μg/ml respectively. There was no significant LMI by cGMP 10^{-4}–10^{-10} M/ml or by cAMP 10^{-4}–10^{-9} M/ml. The ophthalmic "vehicle" without DMS did not produce LMI (not shown). The cells remained viable and excluded trypan blue in all experiments. The migration fan in the absence of steroids is marked by speckling and a surrounding halo whereas LMI induced by DMS results in homogeneous appearing fans (Figs. 2, 3).

The random variation of LMI by antisera to Ig and by DMS was the function of the response of a particular individual. It was not related to the total or differential white blood cell count, to the individual's serum Ig level, or to the Ig chain class against which the antisera were made (10, 11). The scatter of results is thus due to unknown factors, for repeated testing of volunteers led to less than 10% variability.

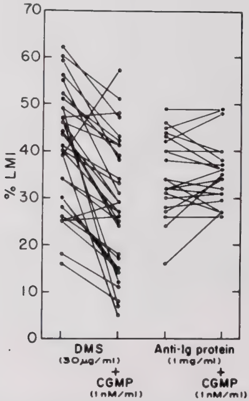

FIG. 1. The effect of cyclic GMP (cGMP) on leucocyte migration inhibition (LMI) induced by 1.0 mg protein from antiserum to various heavy and light chains (Anti-Ig protein) or by 0.6 × 10^{-4}M dexamethasone (DMS-30 μg dexamethasone phosphate).

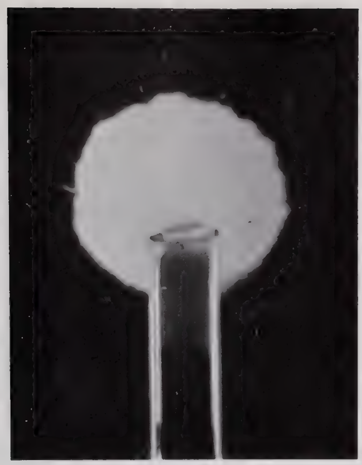

FIG. 2. Leucocyte migration inhibition induced by 0.6×10^{-4}M dexamethasone.

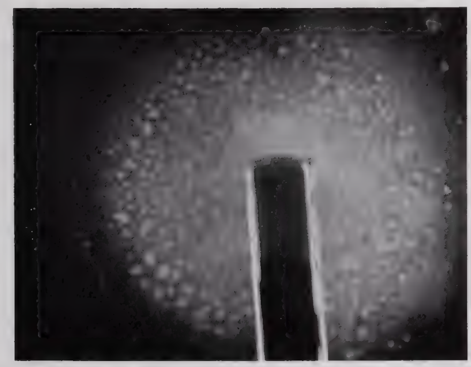

FIG. 3. Leucocyte migration fan in absence of steroid. The speckling and surrounding halo is found in control and in immunoglobulin-induced LMI.

Discussion

In the leucocyte migration inhibition test the individual's white blood cells migrate out of a capillary tube and fan out on a cover glass. Antigen is prepared from soluble or particulate tissue homogenate or from subcellular organelles of cells from tissue homologous with the tissue involved in the patient's disorder. The antigen inhibits the migration of the patient's leucocytes but not of white cells from a healthy individual (1–6). LMI may detect antibody-bearing B cells, monocytes and PMN's and not solely the T lymphocytes which secrete the lymphokines of cell-mediated immunity (10, 11, 13, 16, 22). Incubation of lymphocytes with antigen, in other in vitro tests for cell-mediated immunity, requires less antigen (23).

Antigen stimulates sensitized lymphocytes to secrete migration inhibitory factor (MIF) which inhibits the migration of guinea pig peritoneal cells (23). MIF is thus considered a lymphokine although it is also secreted by B cells and lymphoid and nonlymphoid cell lines (24, 25).

Immunoglobulins identify the B lymphocyte precursors of antibody secreting plasma cells (15). Ig chains as well as antisera to the diverse Ig heavy and light chains and to IgG Fab and IgG Fc can induce LMI in lower concentrations than were used in the present study (10, 11). The leucocytes tested were obtained from healthy individuals not known to be sensitive to homologous Ig or NGS. Antigen induced LMI may result from an antigen-antibody reaction at the cell membrane leading to cellular aggregation and/or from a T cell-B cell-macrophage interaction preventing leucocyte migration (9–11, 13, 19, 26, 27) (Fig. 3). A lymphokine, leucocyte inhibitory factor (LIF) produced by sensitized T and/or B lymphocytes, has been reported to inhibit the migration of PMN's (16, 22, 24). An individual's PMNs may thus act as the target cells for lymphokines released by his own sensitized lymphocytes.

Steroids induced intracellular metabolic changes after reacting with cell membrane determinants and the nucleus. The effects of steroids on leucocyte migration, in vivo and in vitro, may be secondary to these intracellular events (7, 21, 28, 29, 30) (Fig. 2). Antigen-induced lymphocyte stimulation (transformation) and MIF secretion are cellular immune processes diminished by steroids as well as by cyclic AMP (21). Hence the effects of anti-Ig and of DMS on LMI were studied in the presence of cyclic AMP and cyclic GMP. 1 nM cyclic GMP reversed LMI induced by 0.6×10^{4}M DMS (Fig. 1) whereas neither cyclic AMP, or cyclic GMP in other concentrations, influenced steroid-induced LMI (unpublished). It appears that 1 nM cyclic GMP which stimulates neutrophil leucotaxis, interferes with or counteracts the intracellular events leading to LMI induced by DMS (19, 31).

Cyclic GMP did not affect anti-Ig induced LMI, which may result from an antigen-antibody reaction leading to cellular aggregation or the release of LIF (10, 11, 13, 16, 22, 24, 26, 27). The numerous reports in which the LMI test was interpreted as indicative of cellular immunity have to consider that antibody-bearing cells, and not only cells secreting the mediators of cellular immunity, may be detected. In many studies of the same disorders, tissue homogenates, cells and subcellular organelles have been shown to elicit the secretion of lymphokines and antibodies (1, 3, 5, 6, 32–34). The in vitro tests for cellular sensitivity, i.e., lymphocyte stimulation and the secretion of lymphotoxin or MIF, have not always given identical results (23, 24, 35). LMI has correlated as well as other in vitro tests with in vivo skin testing for delayed hypersensitivity (1, 23, 24, 36). A thorough study of the above in vitro tests for cellular immunity is needed for a comparison of antigen dose, incubation time, and the mechanism(s) of lymphokine production and secretion, and it should be correlated with antibody formation.

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Gastrocolic Fistula Complicating Transmural Colitis: Report of a Case and Review of the Literature

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Internal fistulization and abscess formation are common complications of inflammatory bowel disease, but gastrocolic fistulas are rare. Only several cases have previously been reported.

Case Report

A 60-year-old woman (TM #638119) was admitted to Elmhurst General Hospital with a one-month history of right lower quadrant pain.

The patient had intermittent episodes of diarrhea for 20 years, all treated symptomatically. X-ray studies had never been performed. One month prior to admission the patient began to have constant right lower quadrant pain and 6 to 10 loose, mucoid stools per day. There was no history of bad breath, nausea, vomiting, fever, chills or rectal bleeding. She had a 15-pound weight loss in the 3 months prior to admission.

Physical examination revealed a pale, poorly nourished woman. Her abdomen was soft and not distended. There was a firm, tender 15 × 15 cm mass in the right lower quadrant which could be felt on pelvic and rectal examinations.

The patient was afebrile. Her hemoglobin was 9.8 gm%. White blood cell count was 11,300 cells/mm^2 with a shift to the left. Urinalysis was normal. Serum chemistries were normal except for a serum cholesterol of 130 mgm%.

Sigmoidoscopy was normal to 25 cm. A rectal biopsy was taken. A barium enema (Fig. 1) revealed ileocolitis with extensive disease in the ileum, right colon, transverse colon, and splenic flexure. A cologastric fistula was present from the splenic flexure and the greater curvature.

An upper gastrointestinal series revealed a normal stomach without demonstrating the fistula. Small bowel follow-through revealed ileocolitis with matting of small bowel loops in the pelvis and right lower quadrant with ileo-ileal fistulas.

Colonoscopy disclosed a normal rectum and sigmoid colon. At 60 cm there were chronic inflammation and multiple pseudopolyps. Biopsies were taken. The bowel narrowed at this point and the scope could not be passed further.

Gastroscopy showed the stomach to be normal with the exception of an area of ulceration and inflammation along the greater curvature. A biopsy was taken.

Biopsy from the stomach revealed normal gastric mucosa with minimal inflammation. Rectal biopsy was normal. One biopsy from the area of colonic inflammation at 60 cm was reported as "pseudopolyp." The second biopsy was reported as "normal gastric mucosa."

At laparotomy the patient had mild inflammatory bowel disease extending from the ligament of Treitz to the ileocecal valve. The ileum was thickened, dilated and matted in the right lower quadrant. There was extensive serositis and fat wrapping involving the ileum, right colon and the transverse colon. The mesentery was thickened with matted lymph nodes. The colon was adherent to the stomach in the region of the greater curvature.

A resection of the terminal ileum and subtotal colectomy with wedge gastric resection was performed, and an ileostomy and sigmoid mucous fistula constructed. Right Fallopian which was adherent to the ileocecal mass was also excised.

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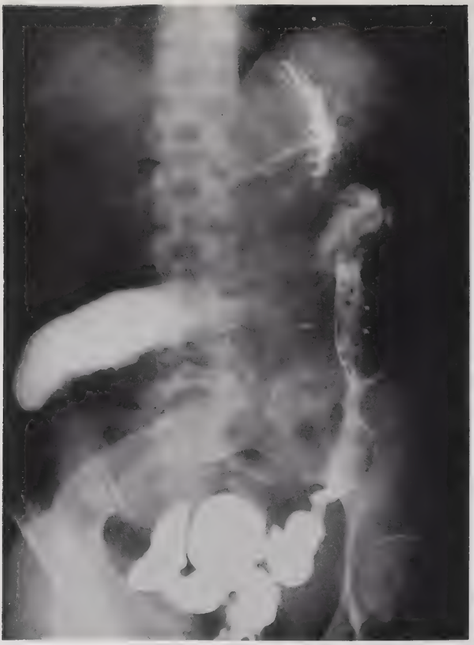

FIG. 1. Barium enema demonstrating colonic pathology and visualizing stomach through cologastric fistula.

The gross specimen revealed extensive ileocolitis with ileo-ileal fistulas and an ileo-cecal fistula (Figures 2a and 2b). A fistula between the cecum and the right Fallopian tube was also present. The transverse colon and the splenic flexure were markedly diseased with extensive linear deep ulcerations and pseudopolyps. The bowel wall was markedly thickened. The mucosa of the colon was denuded and the bowel had a gray, granular lining. The portion of stomach resected was normal. The fistulas are outlined in Figure 3.

Microscopic examination showed:

Extensive areas of ulceration and mononuclear cell infiltration in the submucosa, muscularis and subserosa consisting mainly of lymphocytes, plasma cells and histiocytes. Sections of gastric mucosa showed fundic glands and occasional collection of lymphocytes in submucosa.

Terminal ileum showed inflammation of wall with granuloma composed of histiocytes and occasional macrophages surrounded by lymphocytes.

Diagnosis was granulomatous enterocolitis with fistula formation.

Postoperative course was uneventful and she was discharged 3 weeks after surgery when she had learned to care for her ileostomy.

Six weeks after discharge the patient had gained 10 pounds. Repeat sigmoidoscopy and rectal biopsy were normal. An ileosigmoidostomy was performed. The patient's postoperative course was

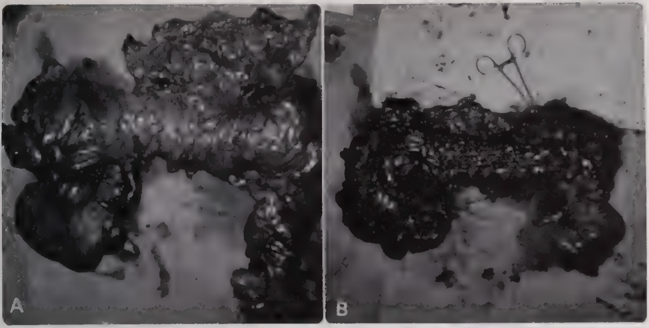

Fig. 2a. and Fig. 2b. Operative specimen, clamp, showing cologastric fistula.

unremarkable. The patient is currently 18 months postoperative and has gained another 10 pounds and feels well. She has 4 to 6 semi-solid stools per day and requires no medication.

Discussion

Gastrocolic fistulas of any cause are uncommon. The usual cause is marginal ulcer following gastroenterostomy (1, 2). In the absence of previous surgery, gastrocolic fistulas may be manifestations of advanced carcinomas of the stomach or colon (3). Rare causes include tuberculosis, cancer of the pancreas, gastric lymphoma, transverse colon carcinoid (3), and diverticulitis of the splenic flexure (4).

Our patient demonstrated typical gross and histologic changes of transmural ileocolitis (5). The bowel wall was markedly thickened and fibrotic. Serositis was prominent. The mesentery was thickened and edematous. Lymph nodes were matted and enlarged. Mesenteric fat wrapping was present. Skip areas were involved, the right colon and transverse colon were markedly diseased but the descending colon and rectum were normal.

Combinations of normal and denuded mucosa were present. The involved mucosa was studded with longitudinal burrow ulcers, serpiginous ulcers, and edematous areas. Transmural inflammation was present. Deep fissures extended from the mucosa to the serosa where microabscesses were prominent. Such deep fissures are reported in 25–30% of large series (6, 7). Adhesions form around these abscesses producing an inflammatory mass and ultimately a fistula.

Fistulas are a common complication of transmural ileocolitis. Most are perianal. However external or internal fistulas occur in 20–30% of patients at some time during the course of the disease (8). Internal fistulas usually arise in diseased loops of small bowel which are matted and adherent. On the other hand, fistulas arising from diseased colon are uncommon (2). Cologastric fistula

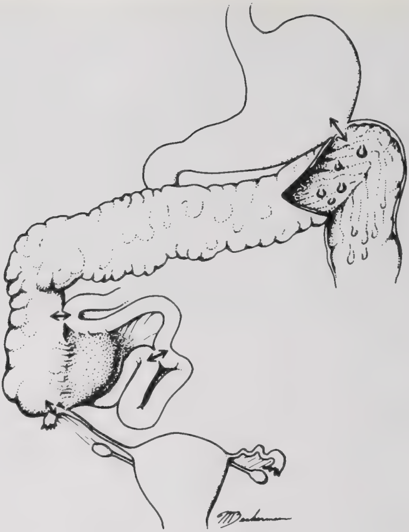

FIG. 3. Diagrammatic outline of fistulas

complicating inflammatory bowel disease is rare and only several cases have been reported (9–11).

In our patient there was considerable inflammation in the region of the splenic flexure at the site of the gastrocolic fistula. There was no evidence of transmural inflammatory disease in the stomach.

As in most cases of gastrocolic fistulas, the fistula was visualized by barium enema, but could not be demonstrated by UGI series. The gastrocolic fistula developed at some time during the course of her illness and was not accompanied by any specific change in her clinical status. She had no loss of appetite and no feculent breath. Interestingly, the colonoscopist went through the fistula and biopsied gastric mucosa. Following subtotal colectomy and partial gastrectomy with subsequent ileosigmoidostomy, the patient has remained clinically well for 18 months.

Summary

A gastrocolic fistula developed in a 60-year-old woman as a complication of transmural ileocolitis. This was treated with subtotal colectomy and wedge gastric resection. The cause and etiology of gastrocolic fistulas are reviewed.

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Benign Mesenchymoma of the Breast

BARRY BENISCH, M.D., BERNARD PEISON, M.D., AND JOHN SARNO, M.D.

Abstract

A benign mesenchymoma composed of smooth muscle, fat, myxoid-chondromatous tissues and cartilage is described. It arose within the upper inner quadrant of the left breast and presented as a painless nodule noted two weeks prior to admission. We are not aware of any previous reports of a tumor of this type arising in the breast.

Introduction

A wide variety of benign and malignant tumors arises in the breast. It is incumbent upon the surgeon and pathologist to familiarize himself with these neoplasms irrespective of their apparent rarity.

Benign mesenchymomas are tumors defined by Stout as neoplasms composed of two or more mesenchymal elements other than fibrous tissue (1). We have recently had the opportunity of examining a benign breast tumor composed of adipose tissue, myxoid-chondromatous tissues and sheets of smooth muscle. To the best of our knowledge this is the first report of a tumor of this type arising in the breast.

Case Report

A 37-year-old white woman had a nodule in the breast first noticed two weeks prior to admission. The mass was in the upper inner quadrant and appeared freely movable. Excisional biopsy was performed.

Pathology. The tumor measured 1.6 × 1.0 × 1.0 cm and was enclosed by a thin rim of fatty breast tissue. Cut section revealed it to be firm with a bulging gray-white surface (Fig. 1). Microscopically, the tumor was unencapsulated and blended imperceptively into the surrounding breast tissue. The tumor was predominantly composed of interlacing bundles of smooth muscle characterized by elongated blunt-ended nuclei and eosinophilic fibers which stained deep red with the Masson-Trichrome stain (Fig. 2). Admixed with the smooth muscle were foci of adult adipose tissue containing areas of myxoid and chondroid tissues (Fig. 3). Frank cartilage was also present (Fig. 4). Mitotic figures were not observed. Osteoid, bone, and striated muscle were not identified. Blood vessels showed neither thickening nor proliferation.

Discussion

Benign mesenchymomas probably arise from pluripotential mesenchymal cells capable of differentiating along varied cell lines. The presence of adipose and vascular tissue, smooth muscle, cartilage, striated muscle, bone and lymphoid tissue have all been described as occurring in these tumors (1, 2). The varied nature of these lesions has suggested to some authors that they represent hamartomas rather than true neoplasms; however, their infiltrative

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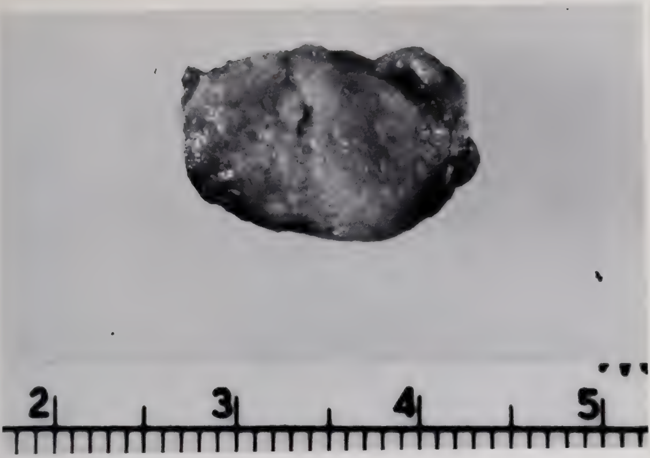

FIG. 1. Gross photograph of tumor showing a bulging cut surface, and peripheral fatty breast tissue.

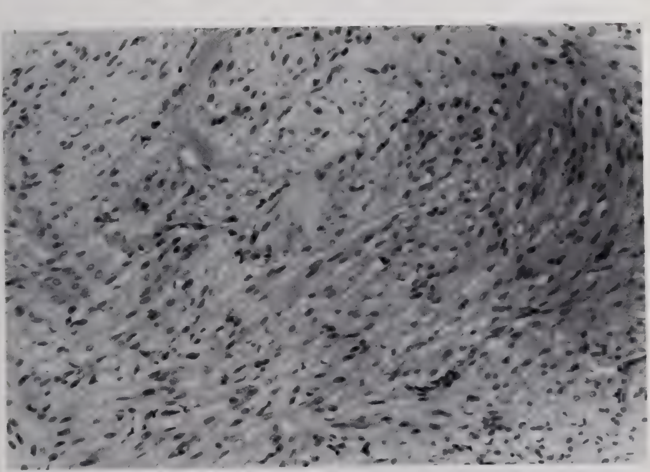

FIG. 2. Tumor showing interlacing bundles of smooth muscle with characteristic plump elongated nuclei (Hematoxylin and Eosin × 100).

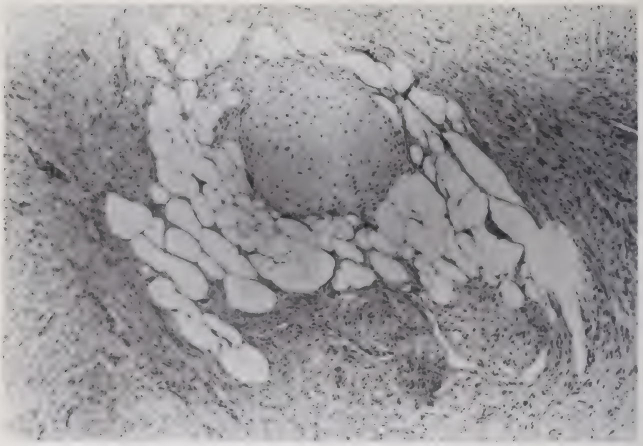

FIG. 3. Focus of adipose tissue with nodule of myxochondroid tissue (Hematoxylin and Eosin × 100).

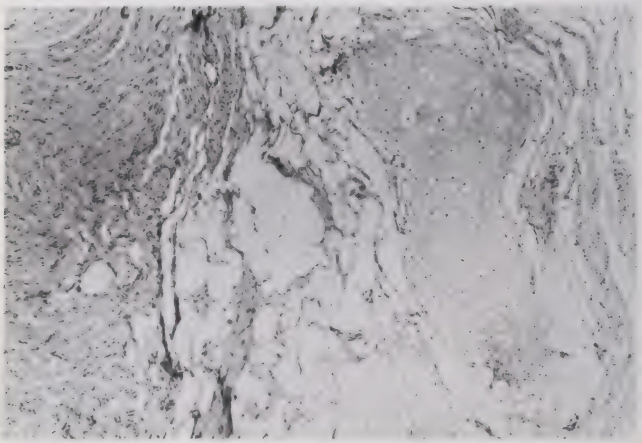

FIG. 4. Focus of adipose tissue with area of cartilage (Hematoxylin and Eosin × 100).

growth pattern, the occasional recurrence, and the existence of a malignant variant (1, 2) all militate against such a hypothesis.

Benign mesenchymomas arise most frequently in the renal and perirenal tissues (1, 2), and have been described as well at such diverse sites as the mediastinum, the extremities, axilla, spinal canal and cord, ileum, and liver (1, 2).

Tumors composed of adult adipose tissue with foci of vascular proliferation have been included within this group by some authors (1, 2, 3) while others prefer the separate designation of angiolipoma (4). These tumors, although uncommon, are far more frequent than their histologically more complex counterparts. Neoplasms of this type, i.e., those composed exclusively of adipose and vascular tissues, have been reported to occur within breast tissue (3). Benign mesenchymal tumors composed of smooth muscle and cartilage as well as adipose tissue have not, to our knowledge, been previously described as arising within the breast.

The tumor in the present case was surrounded by and arose within breast tissue. It was predominantly composed of interlacing bundles of smooth muscle admixed with small foci of fat containing nodules of myxoid, chondroid and frankly cartilaginous tissues. The most likely origin of the neoplasm, in our view, is from a pluripotential mesenchymal cell with subsequent differentiation into smooth muscle, fat and cartilage. The possibility that the tumor represents a vascular leiomyoma with cartilaginous metaplasia of trapped fat, although less likely, cannot be excluded. Vascular leiomyomas have been described, albeit rarely, within the breast but none, to our knowledge, have shown foci of either fat or cartilage (5, 6, 7, 8, 9).

Benign mesenchymomas may be confused with other neoplasms if an inadequate number of sections is taken which reflects only one component of this complex tumor or if the variety of tissues present suggests a more aggressive neoplasm to one unfamiliar with this entity. Complete excision appears to be adequate treatment.

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Subacute and Chronic Tissue Reaction to Metallic Mercury: Two Cases and a Review of the Literature

STEPHEN A. GELLER, M.D.

Abstract

Metallic mercury may, on occasion, be incorporated into tissues and may evoke a pathologic reaction. We have studied two patients in whom this occurred and have reviewed the literature relative to this event. Our two cases demonstrate subacute and chronic tissues reactions to metallic mercury.

Metallic mercury has been recognized as a poison for thousands of years (1). The neuropsychiatric manifestations of chronic mercurialism are well known and are most commonly encountered in industry. Acute mercury poisoning is rare as an industrial problem but may follow intentional or accidental intake of mercury compounds. Metallic mercury may, on occasion, be incorporated into tissues and may evoke a pathologic reaction. We have had the opportunity of studying two patients in whom metallic mercury could be identified macroscopically and have examined the histologic response of the tissue to this metal.

Case Reports

Case 1. A 63-year-old man, who was receiving 5-fluorouracil for metastatic adenocarcinoma of the colon, was admitted to The Mount Sinai Hospital for increasing anemia and gastrointestinal bleeding. He had undergone right hemicolectomy previously and, at that time, peritoneal and hepatic implants were seen. His abdomen was distended, bowl sounds were absent, and shifting abdominal dulness was demonstrated. A long, mercury-weighted intestinal tube was passed in attempt to relieve the small bowel obstruction. During the procedure the mercury-containing bag broke and the patient vomited and aspirated intestinal contents and mercury. Radiograph of the chest showed brightly opaque densities of disseminated mercury throughout both lung fields (Fig. 1). Serum mercury levels rose to 70 μg/ml and urinary mercury levels were as high as 1200 μg/ml. The patient lost all ability to write, had rapid and severe hair loss, loosening of teeth, myoclonic twitching, grimacing movements, and bizarre affect. In addition, he had severe bilateral bronchopneumonia and sepsis, which was complicated by multiple, acute hypotensive episodes. Therapy with N-acetyl-d,1-penicillamine (250 mg/qid) was initially helpful but was discontinued because of another episode of septic shock. Jerking movements and grimacing were no longer present and the tremor was markedly diminished. He continued to be hyperactive and anxious up to the time of death, precipitated by another exacerbation of pneumonia and sepsis, on the 96th day after the aspiration of mercury.

Necropsy. In addition to widely metastatic adenocarcinoma, postmortem examination demonstrated severe bilateral cavitating bronchopneumonia with innumerable scattered droplets of mercury, whch appeared to be surrounded by thin capsules (Fig. 2). Histologically, this surrounding tissue consisted of an inner layer of medium-sized mononuclear cells with compact,

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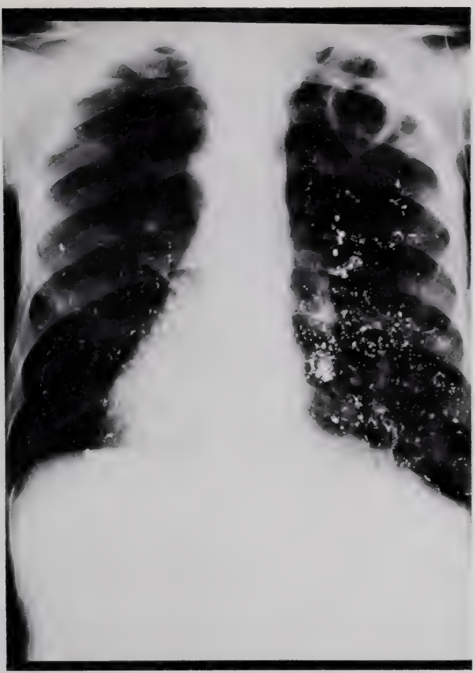

FIG. 1. Case 1. Chest roentgenograph showing mercury disseminated throughout both lung fields, 86 days after aspiration.

central nuclei. Surrounding these cells was an irregular coat of polymorphonuclear leukocytes and granulation tissue (Fig. 3). The kidneys were grossly edematous and microscopically showed tubular dilatation and marked hydropic degeneration of tubular epithelium. The brain was diffusely and severely edematous.

Case 2. A 51-year-old man was admitted to The Mount Sinai Hospital complaining of increasing shortness of breath. A heart murmur had first been heard 7 years before, in 1958, with subsequent development of angina pectoris on exertion and considerable reduction of exercise tolerance. Left heart catheterization, at another hospital, demonstrated aortic stenosis and cardiac surgery was performed in 1960 at which time calcifications were removed from the stenotic aortic valve and commissurotomy performed, with considerable restoration of valve function. He was without symptoms of heart disease for 5 years until rapidly worsening congestive heart failure precipitated the final admission. The heart failure proved intractable and, after an episode of ventricular fibrillation, he died on the 8th hospital day.

Necropsy. The heart was greatly enlarged (660 G) with severe calcific aortic stenosis. The mitral valve had obvious stigmata of rheumatic fever, namely plump, short, fused chordae tendineae and fibrotic deformed leaflets. MacCallom's patch was seen. The lungs were severely

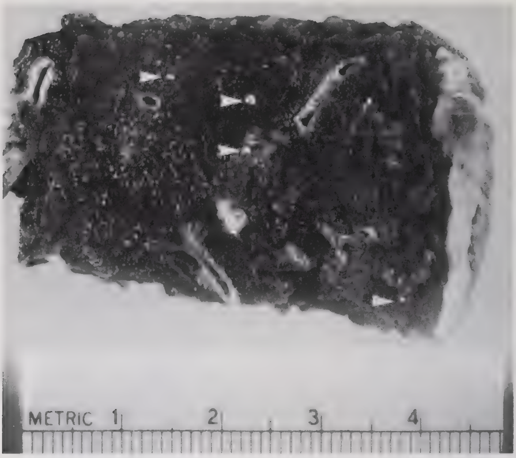

FIG. 2. Case 1. Area of formalin-fixed pulmonary parenchyma showing spherical mercury droplets (arrows).

congested and edematous. The liver was similarly markedly congested and displayed a distinct "nutmeg" appearance. In addition, 1 mm mercury droplets were irregularly scattered throughout the parenchyma, the greatest concentration being close to the capsule (Fig. 4). The droplets were encased by irregular, thin fibrous capsules. Histologically the inner layer of fibrosis was surrounded by a band of lymphocytes (Fig. 5). Similar mercury droplets were identified in one area of the right kidney between adjacent renal pyramids. The parenchyma between the mercury and the capsule was a wedge-shaped, depressed fibrotic area.

Discussion

Metallic mercury has been incorporated into tissues after its use as a cutaneous medicinal (2, 3), after intrabronchial instillation from radiographic studies (4), accidentally during blood sampling (5–8), after accidental aspiration (9), following breakage of long intestinal tubes weighted with mercury (10–16), subsequent to accidental breakage of a clinical thermometer (17–21) or mercury container (22, 23), after self-administration for suicidal (24–26) and non-suicidal (27, 28) reasons, as a consequence of tattooing (29), as well as experimentally (9, 30). Histologic observations were recorded in a number of these cases (2, 5, 6, 10, 11, 13, 18, 19, 21, 14, 16–30). Certain aspects of those reports of human tissue reaction are summarized in Table I.

Necrosis (10, 26) and acute inflammation (10) have been described as early histologic reactions to mercury. Similar findings were documented in experimental studies of dogs given mercury intravenously (9). The central spherical droplets of mercury were found in many of the tissues of the dogs and were

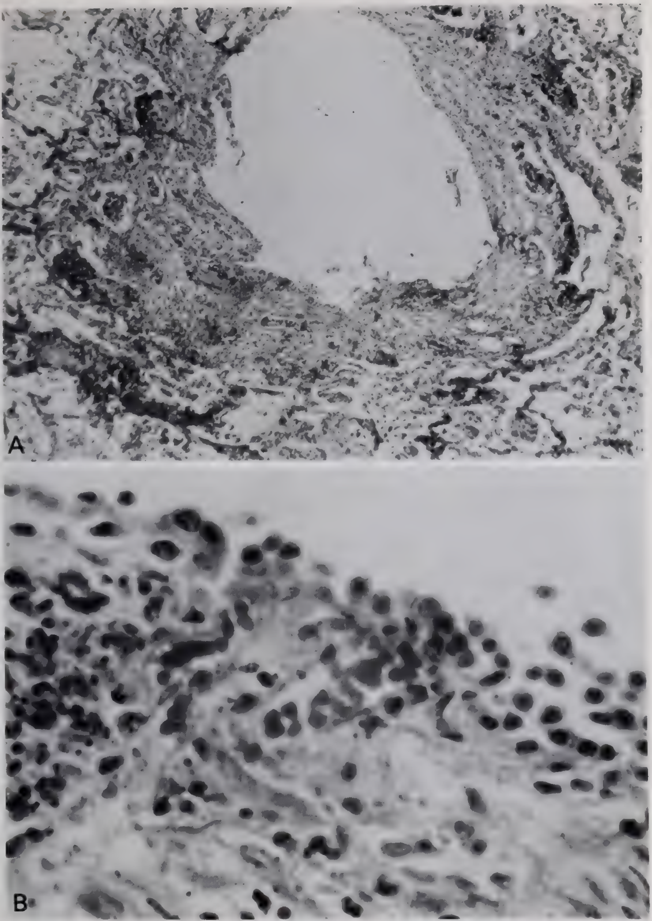

FIG. 3. Case 1. Photomicrograph of pulmonary tissue around mercury space showing inner cost of medium-sized mononuclear cells surrounded by granulation tissue (Hematoxylin and Eosin: A—original × 40; B—original × 400).

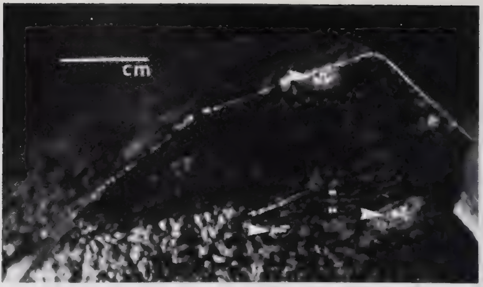

FIG. 4. Case 2. Area of liver showing mercury droplets (arrows) within fibrous capsules.

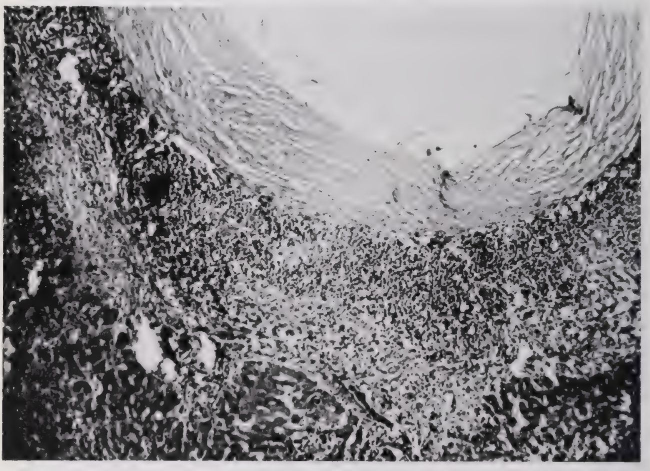

FIG. 5. Case 2. Photomicrograph of liver around mercury space showing fibrous tissue capsule surrounded by band of lymphocytic cells (Hematoxylin and Eosin, original × 100)

surrounded by dense zones of polymorphonuclear leucocytes. This layer of acute inflammatory cells was surrounded by macrophages and granulation tissue.

Granulomatous (2, 13, 18, 19), foreign body giant cell (6, 29) and granulation tissue (5, 13, 27) reactions were found when the mercury had been in tissue for a prolonged period of time, and a fibrous tissue capsule, with surrounding chronic inflammatory cells, may represent the final histologic response to mercury in tissue (11, 18, 19, 21, 24, 27).

TABLE I

Histologic Response to Metallic Mercury in Humans

Author	Reference	Age	Sex	Site of mercury	How introduced	Time in tissue	Toxicity	Histologic response
Rixford	(2)	21	M	Arms, legs	Self, topical medicinal	2 months	No	Epithelioid cells, round cells, polymorphonuclear leukocytes, fibrosis
Lathem	(5)	37	F	Fingertips	Accident, arterial blood sampling	46 days	No	"Proliferative inflammatory reaction", thrombosis with recanalization
Schulz	(6)	4	M	Thyroid	Unknown	Unknown	Yes	Foreign-body reaction
Birnbaum	(10)	28	M	Appendix	Accident, broken mercury bag	Few days	No	Necrosis, hemorrhage, acute inflammation
Lindenmuth	(11)	29	M	Cecal-cutaneous fistula	Accident, broken mercury bag	7 months	No	Lymphocytes, monocytes, fibrous tissue
Crikelair	(13)	12	M	Appendix and peri-appendix	Accident, broken mercury bag	1 year	No	Granulation tissue, epithelioid granulomata, secondary abscesses
Popper	(18)	19	F	Lung	Accident, broken thermometer	4 years	No	Mercury emboli in small pulmonary arteries, bronchiectasis, chronic interstitial pneumonia.
Arcadio	(19)	Adult	F	Hand	Accident, broken thermometer	79 days	Yes	Fibrous tissue
Rachman	(21)	13	F	Finger	Accident, broken thermometer	2 years	No	Dense fibrosis, lymphocytes, histiocytes
Umber	(24)	20	F	Lungs, kidneys, heart, spleen, colon	Attempted suicide, intravenous	4 years	Yes	Fibrous capsule
Johnson	(26)	23	F	Median nerve, muscle	Suicide, intramuscular	31 days	Yes	Necrosis
Hill	(27)	21	F	Skin	Self-administration	Unknown	No	Granulation tissue, fibrosis, chronic inflammatory cells.
Kern	(28)	21	M	Forearm	Self-administration	3 months	No	Acute and chronic inflammation, abscess, granuloma
Lane	(29)	Adult	M	Forearm	Tattoo	1 year	No	Lymphocytes, histiocytes, plasma cells, foreign-body reaction
Geller		63	M	Lung	Accident, broken mercury bag	96 days	Yes	Mononuclear cells, granulation tissue
		51	M	Liver, kidney	Accident, arterial sampling	5 years	No	Fibrous capsule surrounded by lymphocytes

Zahn described infarcts as a consequence of injection of mercury into vessels (30). Evidence of previous infarction was found in the kidney in our Case 2. Hepatic infarction probably did not occur after delivery of mercury to the liver via the hepatic artery. Zahn induced liver infarcts by injecting the mesenteric vein. Further, the usual regeneratory activity of the liver would long since have caused replacement of infarcted areas. Although we are unable unequivocally to document the mode of entry of mercury in case 2, it is likely that the metal inadvertently entered the circulation as a consequence of arterial sampling in preparation for cardiac surgery performed 5 years before death. This complication of anaerobic blood sampling was well known during the period when metallic mercury was used as a sealing medium in syringes (5–8).

Signs and symptoms of mercury poisoning were documented in many of the cases reviewed (6, 19, 24, 26). As expected, toxicity was not related to age or sex (Table 1). It has been suggested that the amount of mercury in tissue is the determining factor (7). This may not be the basis of mercurialism, however, for toxicity has been described after the breakage of a clinical thermometer with introduction of small amounts of mercury into tissue (19, 20). It has been recommended that surgical intervention should always follow contamination with metallic mercury as a prophylactic measure to prevent toxicity, even if only a small amount of mercury is involved. The ionic state of mercury may be of great importance, since mercurous compounds are less lethal than mercuric (31).

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Effects of Lead on the Female and Reproduction: A Review

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Introduction

With increasing numbers of women entering the workplace, attention is being focused on hazards that may have special effects on their health. Lead, especially in relation to the development of an Occupational Safety and Health Administration standard, has received recent attention because of its history of acting as an abortifacient. A survey of the literature has been undertaken: first, to summarize the reported data on lead's effect on human pregnancy; second, to review experimental animal studies of lead's effects on pregnancy; and third, to evaluate the published evidence that women may be more susceptible to toxic effects of lead than men.

Lead has been known to affect women during pregnancy for more than a century. Teratogenesis has been reported and documented by animal experimentation. In addition to lead's direct adverse effect on the course of pregnancy, it has an indirect effect by its toxic action on the male germ cell. There is evidence that women, particularly during specific periods, i.e., adolescence, pregnancy, etc., are more susceptible to toxic effects of lead. Oliver has noted that women are more susceptible to lead poisoning between ages of 18 and 23, and after shorter exposure (1). He noted that women poisoned by lead have a higher incidence of encephalopathy, and a lower incidence of paralysis and colic, than males.

Early Reports

During the late nineteenth and early twentieth centuries, women in the pottery and white lead industries felt lead was an abortifacient. In 1860, Constantin Paul published figures indicating a profound effect of paternal plumbism on sterility and on the viability of the offspring (2). Reid gathered statistics on women in the English potteries and white lead works, reporting that women in lead work, as compared to ones not employed in this work, were more likely to be sterile; and if they became pregnant, to miscarry. If the pregnancy went to term, it was more likely to end in stillbirth; and if the child was born living, death was more likely to come in the first year of life (3).

Pindborg reviewed 25 well-documented cases of mild to moderately severe lead poisoning in Danish women who had ingested lead oxide as an

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abortifacient (3). Sixty percent of the pregnancies in the first trimester ended in abortion. Four pregnancies of five or more months duration resulted in one abortion and three normal infants.

Rennert in 1881 found a high prevalence of convulsions and a peculiar form of macrocephaly in the German village of Almerode where pottery glazing was a home industry (5). He gave the following record of 79 children:

	# Children	Macrocephaly	Convulsions	Stillbirths
Both parents leaded	19	18	17	1
Father leaded, mother slightly	27	17	9	6
Father leaded, mother normal	33	19	13	0
TOTAL	79	54	39	7

Seventy one percent of the children had either macrocephaly or convulsions with a mortality rate of over fifty percent.

In 1908, Chyzzer published an account of a Hungarian village engaged in home pottery glazing where the same peculiar form of macrocephaly prevailed (6). This was confirmed by Oliver (British Inspector of Factories) who made a special journey to Hungary (7).

Torelli in 1930 studied the influence of chronic lead poisoning on offspring of parents in the printing trade in Milan, Italy (3). He found the abortion rate for Milan in general to be 4–4.5%, but among the wives of printers the rate was 14% and among the women printers 24%. The average death rate in 1930 in all Italy for children during the first year was 150/1,000 births, but for this group it was 320/1,000.

Teleky analyzed German female workers in the printing industry, and stated that spontaneous abortions were three times as common among those exposed to lead as those not exposed (8).

Arlidge in England, reporting on 71 females working with lead after marriage, found that 11 percent of pregnancies ended in miscarriage (9). The neonatal mortality was almost 40 percent.

Tardieu reported to the French government in 1905 that 608 out of 1,000 pregnancies in lead workers ended in abortion (9).

Legge, in summarizing the reports of 11 English factory inspectors in 1897, found that of 212 pregnancies in 77 females working with lead, only 61 living children were produced (10). Fifteen had never become pregnant; there were 21 stillbirths; miscarriages occurred ninety times; and of 101 children born, 40 died in their first year. Legge also noted that when pregnant animals were fed lead, they always aborted. Legge felt that lead affected pregnancy directly through the female's exposure to lead; he found little evidence to suggest any adverse effect on pregnancy or the health of the offspring if only the male were exposed to lead.

However, in 1905 Rudeaux analyzed 442 pregnancies in women married to lead workers; 66 ended in abortion, and 241 in premature birth (3). Also in 1905, Deneufbourg noted 23 abortions and stillbirths amongst 134 pregnant females exposed to lead (11). He found a 25.5 % (113 in 442 pregnancies) abortion and stillbirth rate in the wives of male lead workers. He also noted that in animal experiments, offspring of leaded parents had lead far more evenly distributed in the various bodily organs than in mature individuals.

In an excellent study from Japan using an unexposed control group, Koinuma compared the marital life records of workmen exposed to lead in storage battery plants with the records of those working in nonleaded occupations (1). The sterile marriages constituted 24.7 % for the lead group and only 14.8 % for the nonlead group. The percentage of pregnancies ending prematurely or in stillbirth was 8.2 for the lead group and 0.2 for the control group.

In 1911, Oliver published statistics on the effect of lead on pregnancy:

	# abortions and stillbirths per 100 females	# neonatal deaths (first year) per 100 females
Housewives	43.2	150
Female workers (mill work)	47.6	214
Female exposed to lead premaritally	86.0	157
Female exposed to lead after marriage	133.5	271

A more recent report on occupational lead exposure and pregnancy comes from Nogaki in Japan (12). A detailed study of the pregnancies of 104 Japanese women before and after beginning lead work showed an increase in miscarriages to 84.2/1,000 pregnancies from a prelead rate of 45.6/1,000. The miscarriage rate for 75 comparable employees not exposed to lead was 59.1/1,000 pregnancies. The maternal blood leads ranged from 0.110 mg % to 0.317 mg %.

Gilfillan (1965) has presented an interesting hypothesis that the fall of Rome was caused by sterility in the ruling class due to their consumption of wine sweetened with lead (13).

Thus, it was reported as early as the 19th century, that lead had a damaging effect on fertility, the course of pregnancy and the development of the fetus. It was also apparent that exposure to lead by either sex could lead to reproductive failure. Certainly, in these early reports, the lead exposures were uncontrolled, and may have been very high.

Recent Studies

More recently, A. T. Wilson in Scotland reported the effects of abnormal lead content of water supplies on maternity patients (14). He noted that the water supplies of his district often exceeded the World Health Organization standard limit for the lead content of drinking water (0.05 mg/l) due to the soft water's corrosive action on lead piping. He analyzed routine prenatal urine specimens for coproporphyrin in 72 patients during 75 pregnancies over a two-year period. He divided his patients into two groups: one with unrestricted access to the water supplies, and one encouraged to drink milk and restrict water. Thirty-one percent (11 of 35) in the unrestricted group reached a level of coproporphyrinuria exceeding 100 μg/l versus one out of forty in the restricted group. Five pregnancies in four patients in the former group were abnormal:

1. Intrauterine deaths of fetus at 33 weeks. Maternal blood leads were 55, 34 and 72 μg/100 ml at weekly intervals after fetal death, and 51 μg/100 ml two months later. Analysis of fetal tissues for lead were: 1) Kidney 0.2 mg/100 G (normal 0.01-0.16 for normal adults by Kehoe) and liver 0.53 mg/100 G (normal 0.04-.28 mg/100 G for normal adults); 2) Congenital defects (nystagmus, albinism, and hemangioma). Maternal blood lead was 31 mg/100 ml; 3) Congenital defects (multiple hemangiomata). The mother had been sterile for six years previous to this pregnancy which had a threatened miscarriage at 11 weeks and subsequently delivered prematurely; and 4) Two abnormal pregnancies occurred in a woman with four previous miscarriages and two surviving children. The first included a threatened miscarriage at seven weeks with a maternal blood lead of 53 mg/100 ml and subsequently an antepartum hemorrhage occurred at 36 weeks with premature delivery.

Lead has been found in concentrations in umbilical cord blood of newborns parelleling that of the mother (15, 16). Gershanik et al in examining 98 cord blood pair samples found a coefficient of correlation of 0.6377 with maternal samples ($\bar{x}$ 10.1 μg/100 ml newborns and 10.3 μg/100 ml mother) (16). Lead has also been found to be transported into maternal milk during lactation.

Fahim et al compared the course and lead values in 249 pregnancies in Columbia, Missouri, with 253 occurring in the center of America's lead belt at Rolla, Missouri (18). At Columbia, greater than 96 % delivered normally at term, 3 % were preterm (defined as a neonate born before 37 weeks of gestation and weighing less than 2500 grams), and less than 1 % had premature rupture of the membrane (occurring in an insulin-dependent diabetic). At Rolla, only 70 % were term, 17 % had premature membrane rupture (defined as spontaneous rupture of the membrane before the onset of labor and when labor does not begin within 12 hours), and 13 % were preterm. The striking blood lead findings from the Rolla lead belt area were a doubling of maternal blood lead in the premature membrane rupture and preterm groups (4.6 ± 0.08 μgm/100 gm to 14.2 ± 0.78 and 17.5 ± 1.06 respectively). There also was a three- to fourfold increase of fetal blood lead in the premature membrane and preterm groups.

Palmisano et al reported a case of a ten-week-old infant with evidence of neurologic defects, intrauterine growth retardation and postnatal failure to thrive which was the conceptus of a 33-year-old woman with a history of long-term ingestion of "moonshine" whiskey (19). After challenge doses of calcium EDTA, the infant and the mother each excreted an abnormally large amount of lead in the urine. A recent survey based on chemical analyses of untaxed "moonshine" whiskey samples disclosed that 53 percent contained concentrations of lead above 1000 μg per liter.

Greenfield reported a patient with plumbism in 1957 who subsequently became pregnant and had a normal pregnancy productive of a seven-pound normal infant (20). During the pregnancy, the blood and urine tests for lead were normal (less than 10 μg/100 gm. and 0.11/mg/liter respectively).

A family lead-poisoned by heating their home with lead battery casings was described by Angle and McIntire (21). The mother was eight months pregnant, and had a blood lead of 0.240 mg % with signs and symptoms of lead poisoning. She was treated with a seven-day course of intravenous calcium disodium edetate 75 mg/Kg/day. A month later she delivered a normal infant weighing 3200 grams. On a four-year follow up, pediatric, neurologic, and developmental assessments of the boy were normal.

Abendroth described a similar case in Germany of a woman in her fifth month of pregnancy who took approximately 25 grams of an inorganic lead compound (probably lead oxide) over a period of about three weeks (22). Following aggressive chelation therapy, a healthy baby was born and was reportedly doing well after four years of followup.

The reproduction ability of men occupationally exposed to lead is interfered with by altered spermatogenesis. Lancranjan et al reported a significant increase in teratospermia amongst lead-poisoned workmen (blood lead mean 74.5 μg/100 ml) and workmen with moderately increased absorption (blood lead 52.8 μgm/100 ml) (23). Hypospermia and asthenospermia were increased not only in both preceding groups, but also those with only slightly increased absorption (blood lead mean 41 μgm/100 ml). They also determined urinary gonadotropins and 17-ketosteroids which did not differ from controls. Thus, they concluded that lead has a direct toxic effect upon the male gonads. Abnormal spermatogenesis may be the cause of the high fetal wastage rate amongst the wives of lead workers.

Soviet investigators had previously demonstrated the effects of lead in causing sperm damage in animals (24). They also stated that among the effects of a parental poison burden in children, functional disturbances prevail by far, ahead of mutations and malformations.

Pregnancy is a stress which may mobilize lead from skeletal storage sites. Both iron deficiency and calcium deficiency increase the susceptibility to lead toxicity; women have an increased risk of both deficiencies during pregnancy, and postpartum. The cause of the increased perinatal mortality may be lead's mutagenic and teratogenic effects (25–28). Important genetic effects may be latent for several generations and, in addition, the damage may be permanent. Experimental animal studies with lead have demonstrated increased rates of developmental anomalies, chromosomal damage, and decreased rates of pregnancy.

Experimental Investigations

Varma et al fed 2 % lead acetate to 14 male Swiss mice for four weeks (29). He then allowed each to mate for one week with three virgin untreated females. The overall incidence of pregnancy, indicative of fertility, was 52.7 % in the control group, as compared to 27.6 % in the treated group. The fertility of the treated males was reduced by 50 %. He calculated the mutagenicity index (number of early fetal deaths/total implants) to be 10.4 for lead-treated mice versus 2.9 for controls ($x^2 = 10.4$ $p \geq .05$).

A three-generation, six-litter rat reproductive study showed no effect of dietary lead at concentrations of 0,10,50,100,1,000 and 2,000 ppm on the number of pregnancies, number of pups born alive, fertility index, gestation index, viability index, or lactation index (30). The average weight of rats fed the two higher concentrations was slightly decreased. In contrast, Schroeder et al administered 25 ppm lead in the drinking water to pregnant mice and rats, observing runting reproductive failure, and shortened life span.

Weller fed commercial white lead in capsular form to guinea pigs (27). He noted that the offspring from leaded males and nonleaded females had significantly lower birthweights (81.5 gm versus 66.3 gm) compared to those of nonleaded parents. There was also a high rate of mortality of guinea pigs in the first week after birth when the father had been fed lead.

Hilderbrand et al fed lead acetate at doses of 5 and 100 micrograms orally to 80 male and female rats for 30 days (32). At the end of the study period, the blood leads of the females were higher than the males: 30 μgm/100 ml vs 19 μgm/100 ml at 5 μgm lead acetate, and 53 μgm/100 ml vs 30 μgm/100 ml at 100 μgm lead acetate. They noted impotence and prostatic hyperplasia in the males at the lower dose, progressing to testicular damage in those reaching blood leads of 50 μgm/100 ml. In the females, they noted irregularity of the estrus cycle at both doses. They also demonstrated an inhibitory effect by lead upon hepatic microsomes at both doses in both sexes. Electron micrographs of the liver demonstrated a decrease in free ribosomes and smooth endoplasmic reticulum in the treated rats. Sleeping times following 15 mg of pentobarbital per Kilogram were prolonged in both treated groups compared to controls. Thus, they concluded that lead inhibited hepatic detoxification mechanisms in both female and male animals.

Ferm and Carpenter produced specific congenital-skeletal malformations (sacral and tail vertebrae) in hamster embryos following the treatment of the pregnant hamster with various salts of lead (33). They did not evaluate the possible morphologic changes of dead or resorbing embryos, where the very severe developmental anomalies were likely to have occurred. Ferm has also reported that in the presence of cadmium, the teratogenic effect of lead in hamsters is potentiated (34).

Karnofsky and Ridgwey injected lead nitrate into the yolk sac of the chick embryo (35). They found that this produced a severe, characteristic injury to the central nervous system; widespread brain hemorrhage was noted to be followed by necrosis and hydrocephalus. The central nervous system injury occurred after injections from the third to the ninth days of embryogenesis; it was thought that on the earlier days the circulatory system to the brain was less well-developed. Gilani demonstrated congenital cardiac anomalies by studying 8-day-old chick embryos which had been administered lead acetate on the second day of incubation (36). The incidence of cardiac anomalies rose with increasing doses of lead. Other important anomalies were: retardation of body size, micromelia (malformation of the limbs), shortened neck, microphthalmia, ruptured brain, shortened beak, twisted neck and limbs, and everted viscera (37)

Stowe et al fed 1.0 % lead acetate to white leghorn female hens from age four weeks onward (38). The lead-toxic hens grew more slowly, reached sexual maturity later, and laid significantly fewer eggs than control hens. One-fourth of the eggs laid by the lead-toxic hens had soft and malformed shells.

Chromosomal Aberrations

Muro and Goyer analyzed chromosomes from leukocyte culture of mice fed a diet of 1 % lead acetate (39). They observed an increased number of gap-break type aberrations, largely involving single chromatids. The chromosomal abnormalities included 28 (16.9 %) gaps vs 4 (1.3 %) in the controls, 9 (5.4 %) breaks vs none in the controls, and 20 (12 %) fragments vs none in the controls.

Schwanitz et al reported a three- to fourfold increase in chromosomal aberration in human lymphocytes treated in vitro with lead acetate as compared with control cells exposed to sodium acetate (40). He found an increase in chromosomal aberrations (breaks and gaps) in the peripheral blood lymphocytes of eight male workers in a lead oxide factory; also, he noted a correlation between percentage of abnormal mitoses and excretion of delt-amino-levulinic acid in the urine.

In contrast, O'Riordan and Evans reported the results of chromosomal analysis of 66 shipyard workers, finding no differences in chromosomal aberrations between shipyard workers known to be exposed to lead and other shipyard employees (41). There was no difference in the chromosomal analysis between the groups according to blood lead 40, 40–80, 80–120 and 120 μg/100 ml) or according to job category. Nevertheless, they did find a significant increase in all of the shipyard workers' chromosomal abnormalities (breaks and gaps) compared to individuals in the general population, not employed in shipyards. They emphasized the technical difficulties in chromosomal analysis, and stressed the importance of analyzing the culture after forty-eight hours of growth to prevent the cells from entering a second mitosis which increases the natural occurrence of gaps and chromatid aberrations. Bauchinger and Schmid compared twenty industrial workers with exposure to lead dust and twenty-nine policemen with increased lead absorption to a nonexposed control group, and also found no increase of chromosomal aberration after forty-six hours of culturing lymphocytes (42).

Lehnert in Germany, found an increase in gap-break chromosomal changes in lead workers having a blood lead in the range of 62–89 μg/100 ml (43). He found a positive correlation with an increased urinary ALA and the percent of abnormal mitoses seen. Lead acetate solutions (10^{-4} to 10^{-6}M) added to normal leukocyte cultures produced the same chromosomal abnormalities.

The data of Lehnert and Schwanitz have been corroborated by Forni and Secchi in Italy (44). They analyzed chromosomes from cultures of blood lymphocytes from 65 workers occupationally exposed to lead, and in 65 unexposed controls matched for age. The workers were subdivided into three groups: Group I, 15 workers with preclinical intoxication (blood lead mean 64.07 ± 16.69 μg/100 ml), Group II, 37 workers with clinical poisoning (blood lead mean 77.73 ± 24.89 μg/100 ml), and Group III, 13 workers with past lead poisoning, no longer exposed to lead for at least 18 months (blood lead mean

55.62 ± 16 μg/100 ml). There was a significantly increased rate of chromatid aberrations and of unstable chromosome changes in both Groups I and II, when compared to controls, but the differences were not significant for Group III. Culture time was reported 68–70 hours. In a subsequent discussion, Forni stated that they did not find significant differences in rates of unstable chromosome abnormalities between 48–50 hour cultures and 68–70 hour cultures. Lymphocytes from controls as well as lead-exposed workers were both cultured for 68–70 hours.

There is further evidence that women may be more susceptible than male coworkers to the toxic effects of lead. Bell et al reported that when intravenous lead compounds were used during the early part of this century in the therapy of cancer, women exhibited symptoms at 40 mg while 100 mg was necessary to demonstrate the same symptoms in men (45).

Seppalainen found subclinical neuropathy (decreased motor conduction velocity, particularly of the slow conducting fibers of the ulnar nerve) in lead workers with blood leads consistently less than 80 μg/100 ml (46). She noted that women were highly susceptible to these neuropathic effects; for example, three out of four women studied also had fibrillations.

Stuik and Zielhuis administered lead acetate (20 μg/Kg bodyweight/day) for three weeks to human volunteers of both sexes; a significant increase of protoporphyrin IX content of the erythrocytes was noted in female subjects only (47). The blood lead level increased in both groups of volunteers.

Klevay has reported that lead levels in the hair of 242 females were significantly higher than those of 184 males (median 17.9 μg/G vs 11.4 μg/G $p \leq .0001$) (48).

The difference between the sexes may be due to hormonal effects on drug metabolism, for example, testosterone has a stimulatory effect on microsomal drug metabolism. Hepatic microsomes from male rats have been reported to be able to metabolize many drugs three to four times more rapidly than those from females (49). This sex variation in microsomal metabolism is absent in immature animals, and is eliminated by castration or injection of the antagonistic steroid.

Recent reviews of lead have focused on the clinical picture of lead poisoning occasioned by sporadic outbreaks, subclinical effects of lead from occupational exposure, childhood lead poisoning from lead-containing paint in older buildings and environmental contamination leading to an increased body burden of lead (50, 55). The role of lead's toxic effect on pregnancy and reproduction has received little notice. That this is unlikely to continue was emphasized in a recent editorial on lead poisoning and decreased fecundity in men (56).

Conclusion

Biologic evidence is available indicating that women may be more susceptible to toxic effects of lead. In several animal models, lead exerts a profound noxious effect on pregnancy and fetal development. In addition, the older history of lead's use in the workplace amply documents a deleterious effect on human reproduction. Whether lead is responsible for chromosomal abnormalities in exposed workers remains an unresolved issue. However, there may be no threshold limit at which adverse effects could not occur in the course of development of the human fetus.

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The Sebaceous Duct Adenoma (So-Called Syringoma)

JOHN KWITTKEN, M.D.

Syringoma, a term derived from the Greek word *syrinx* meaning a tube, has been accepted almost universally as signifying a benign tumor of eccrine sweat apparatus origin (1–8). This belief has been strengthened not only by the resemblance of the ductal structures formed by the neoplasm to eccrine glands, but also by histochemical reactions similar to that observed in normal eccrine structures. Some observers, however, are not convinced of the eccrine nature of these lesions and make strong inferences indicative of apocrine origin, a formerly favored concept (9). After studying a number of these lesions, it is my firm conviction that they bear no etiologic relationship to either sweat apparatus and are most likely of sebaceous duct origin.

Materials and Methods

Skin biopsies of 25 lesions showing characteristic clinical and histologic features of so-called syringomas were reviewed from the files of The Skin and Mucous Membrane Biopsy Laboratory. The majority of lesions involved the eyelids, but other sites included the forehead, nose, cheek, neck, and abdomen. The patients' ages ranged from 11 to 79 years and 61% of the cases occurred in women. Staining techniques utilized included Hematoxylin and Eosin, periodic acid-Schiff with and without diastase, and Verhoeff's method for elastic tissue.

Results of Case Study

All lesions showed similar microscopic features. Involving predominantly the upper half of the corium, one observed variable numbers of small ductular structures generally containing eosinophilic flocculent material within their lumens. Their walls were composed of from two to several layers of uniform epithelial cells, the innermost layer or layers appearing cuboidal with vacuolated cytoplasms and the outermost layer or layers appearing basaloid. Occasional ductular structures containing laminated keratin within their lumens and lined by partially compressed and keratinized stratified squamous epithelium showing a granular layer were also encountered (Fig. 1). The adjacent stroma appeared sclerotic and contained nests and cords of uniform

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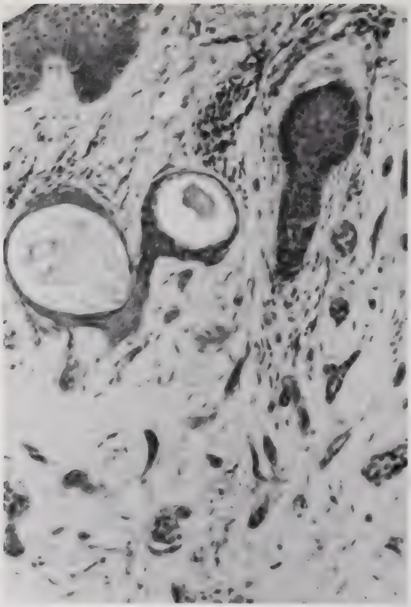

FIG. 1. At left of center, one sees a short cord of uniform basaloid cells connecting a ductular structure (on right) having a luminal layer of vacuolated cuboidal cells with flocculent material within its lumen to a ductular structure (on left) lined by compressed stratified squamous epithelium with a granular layer containing laminated keratin within its lumen (H & E × 250).

basaloid cells in variable numbers, many of which were connected to the basaloid cells forming the outermost layer of the ductular structures formerly described. In some instances, these latter ductular structures seemed to merge with small, immature-appearing sebaceous gland lobules (Fig. 2). No communication with eccrine, apocrine, or pilar structures could be demonstrated in any of the cases studied. As noted by Pinkus and Mehregan (9), the sclerotic stroma showed clumping of elastic tissue fibers in the majority of cases (Fig. 3).

Variable amounts of glycogen were observed within most of the epithelial cells forming this tumor and PAS-positive diastase-resistant material was present within the lumens of many of these ductular structures as well as within some of the vacuolated cells lining them. No basement membrane could be demonstrated about the cords of basaloid cells and ductular structures with the periodic acid-Schiff reaction. Variable amounts of glycogen were also noted within the epithelial cells of normal sebaceous and sweat glands, including their respective ducts. In addition to a PAS-positive diastase-resistant basement membrane about these normal glands and ducts, similar staining material could be demonstrated within the lumens of some sebaceous ducts, eccrine ducts, and secretory portions of eccrine glands as well as within some of the cells lining them.

Discussion

The sebaceous duct is lined by a stratified epithelium showing epidermal keratinization, the number of layers of which decreases toward the acini. It represents a prolongation of the outer hair sheath. The epithelial cells of the sebaceous duct normally produce keratin and sebum (10), particularly those cells forming that part of the duct nearest the acini (11). Therefore, a benign tumor developing from the anlage of the sebaceous duct would be expected to produce a neoplasm showing the histologic features of the so-called syringoma. Since these cells have bimodal differentiating potentialities, one might expect

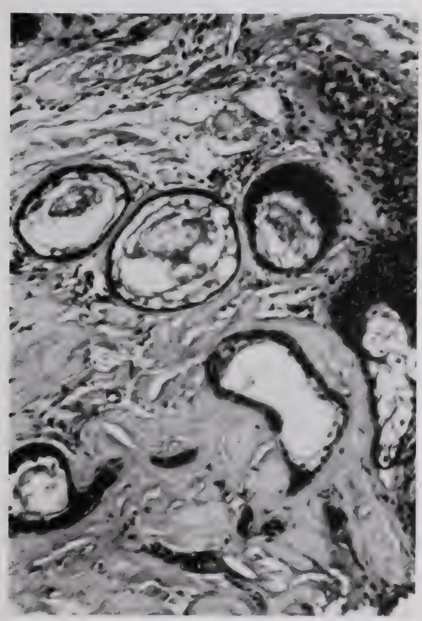

FIG. 2. This shows what seems to represent multiple cross-sections through a characteristic ductular structure merging with a small immature-appearing sebaceous gland lobule at the right margin (H & E × 250).

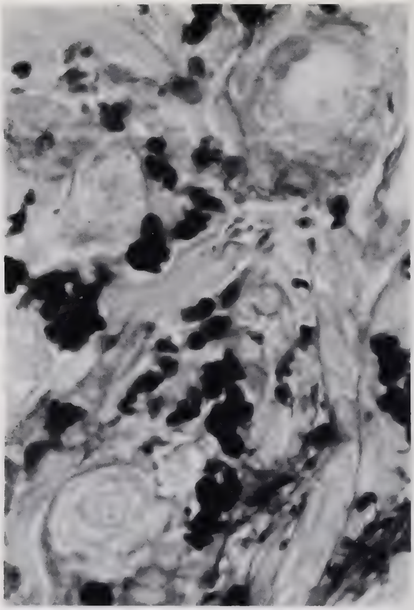

FIG. 3. Note conspicuous coarse clumped elastic tissue fibers within the sclerotic stroma (Verhoeff's elastic tissue stain × 400).

to find, in addition to the usual well-known components, keratin-filled ductular structures lined by stratified squamous epithelium and/or sebaceous gland lobules forming an integral and occasionally a conspicuous component of some lesions. Indeed, such is the case. In fact, it is surprising that apparently no lipids have been demonstrated within the vacuolated cells of these tumors thus far, either by the electron microscope or histochemical methods. Studies are currently in progress for this determination and will be the subject of another report.

The histologic enzymatic evidence linking these tumors to an eccrine origin (3, 4) is, in fact, inconclusive and controversial. In addition to normal eccrine sweat glands and sebaceous ducts showing similar staining reactions with the periodic acid-Schiff technique, most of the enzymes observed within these lesions are also present in substantial amounts within normal sebaceous glands (2, 11, 12) including succinic dehydrogenase, indoxyl esterase, aminopeptidase, B-glucuronidase, and phosphorylase. In agreement with Pinkus and Mehregan (9), I also feel that histochemical evidence should not be as rigid as it is proposed to be in the field of cutaneous neoplasia.

Even the clinical features strongly tend to negate the etiologic relationship between these lesions and eccrine structures. Not only do these tumors always develop at sites where sebaceous glands normally occur, but also they have never been observed on the palms and soles where sebaceous glands are not normally present and where eccrine glands are most numerous (11). Thus, since one appears to be dealing with a benign neoplasm that forms ductular structures and contains other components simulating the normal sebaceous duct, I believe that they develop from sebaceous duct anlagen. It is therefore felt that the appellation syringoma is a misnomer and that it be replaced by the term sebaceous duct adenoma.

Summary

Skin biopsies of 25 so-called syringomas were reviewed. No communication with eccrine, apocrine, or pilar structures could be demonstrated. Since these lesions form ductular structures and contain other components simulating portions of the normal sebaceous duct, it is felt that they develop from sebaceous duct anlagen. It is therefore proposed that the appellation syringoma is a misnomer and that it be replaced by the term sebaceous duct adenoma.

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Pancreatic Pseudocyst: Is Endoscopic Retrograde Cholangiopancreatography Contraindicated?

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Abstract

A case of pseudocyst of the tail of the pancreas complicated by sepsis and infection of the cyst following endoscopic retrograde cannulation of the pancreatic duct is reported. Emergency surgical intervention was required. Asymptomatic hyperamylasemia and hyperamylasuria are well documented and invariably benign. Retrograde study of the pancreatic duct for suspected pseudocyst can yield reliable information. However, serious septic complications, as demonstrated by this case, can ensue and this consideration should caution against routine use of this diagnostic tool in this disease. If retrograde cannulation is performed, prophylactic antibiotics should be instituted and surgical consultation obtained.

The value of endoscopic retrograde cholangiopancreatography (ERCP) as a diagnostic aid in pancreatic and biliary disease has been repeatedly demonstrated (1–3). Complications directly attributable to ERCP have been reported in approximately five percent of those patients studied (2–5). There may be several groups of patients in whom the frequency of complications is greater. Zimmon (4) has recently reported that the incidence of complications following ERCP is significantly higher when there is pancreatic disease or extrahepatic biliary tract obstruction. In his report, four of the eight duct-injection-related complications occurred in patients with relapsing pancreatitis. Davis (5) has reported one patient with a pancreatic pseudocyst in whom septic complications followed ERCP.

We present a case report which demonstrates the septic implications subsequent to ERCP in a patient with pancreatic pseudocyst.

Case Report

J. B. is a 37-year-old alcoholic man with a three-month history of periumbilical and left-sided abdominal pain exacerbated by alcohol intake, and a 15-pound weight loss over the same period of time. Because of recent anorexia, nausea, and vomiting, an upper GI series was obtained. This demonstrated a retrogastric mass (Fig. 1) and the patient was admitted to the hospital. The patient was afebrile and pertinent physical findings included mild epigastric and left upper quadrant tenderness, guarding and fullness, and minimal hepatomegaly. Liver function tests, white blood cell count, and amylase were all normal. Abdominal x-rays showed no abnormal calcifications in the area of the pancreas.

Intravenous pyelogram and nephrotomogram (Fig. 2) and barium enema showed evidence of a large mass in the tail of the pancreas, confirmed to be cystic by ultrasonography. The patient's condition remained stable while these studies were obtained.

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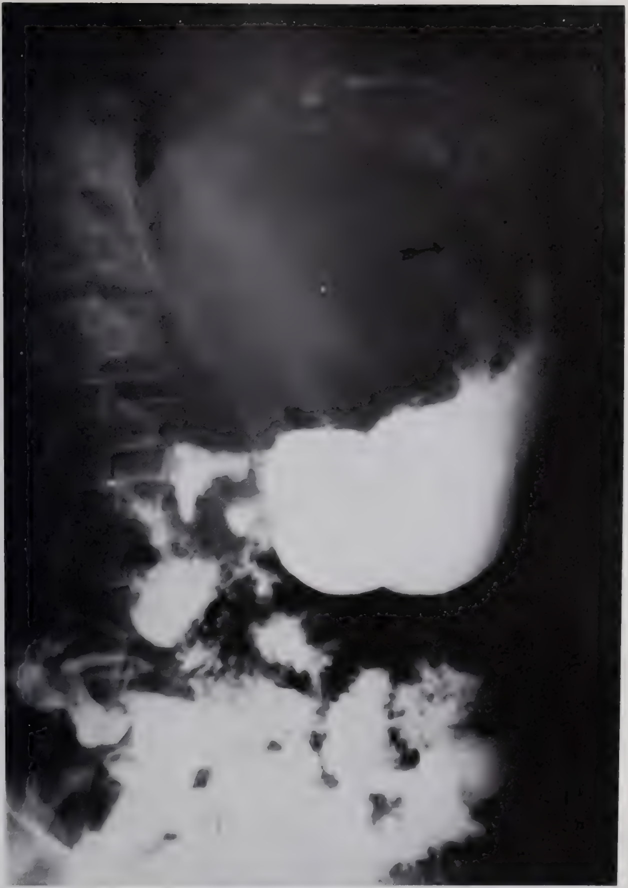

FIG. 1. Upper gastrointestinal series demonstrating anterior and lateral displacement of the stomach (arrow).

On the tenth hospital day, endoscopic retrograde cholangiopancreatography (ERCP) was performed. The common bile duct filled normally. The pancreatic duct was of normal caliber; however, there were dilated tertiary branches which communicated with a large pseudocyst of the tail of the pancreas (Fig. 3). Twelve hours later, the patient experienced upper abdominal pain

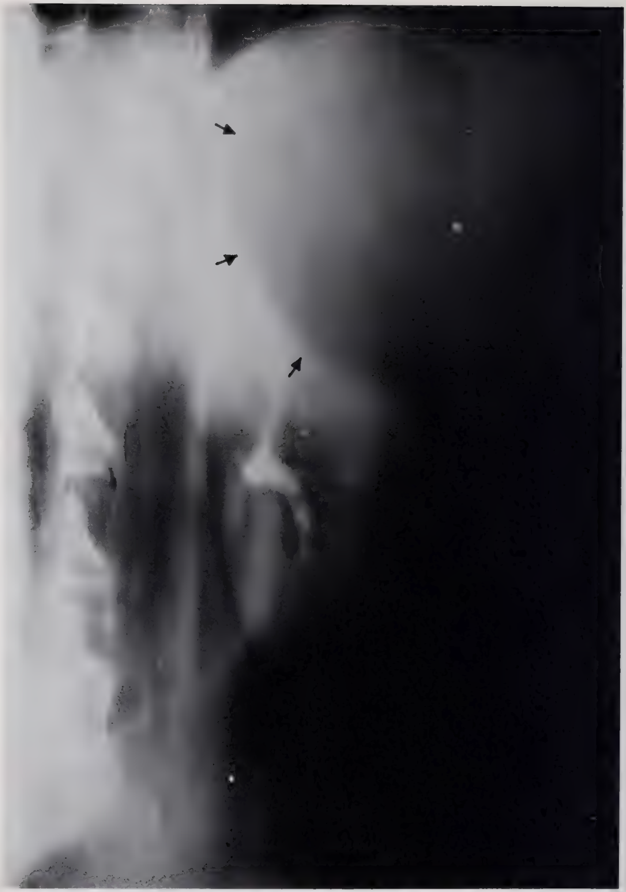

FIG. 2. Nephrotomogram demonstrating smooth cystic mass (arrow) superior to the left kidney.

with increased tenderness and guarding in the left upper quadrant and decreased bowel sounds. Temperature rose to 101 degrees F. White blood cell count rose to 18,700 cells per mm^3, but amylase remained within normal limits. Despite treatment with intravenous antibiotics and nasogastric suction, the patient remained febrile and the abdominal findings worsened during the next twenty-four hours. Operation was performed 48 hours after the endoscopic study and a large infected pseudocyst of the tail of the pancreas was drained by performing a pancreatocystogastrostomy. A sump tube was placed in the pseudocyst, through the anastomosis, and then out the anterior wall of the stomach. A separate gastrostomy was also performed (Fig. 4). Culture of the cyst fluid grew Serratia marcescens and Pseudomonas aeruginosa. The patient made a satisfactory recovery and was discharged on the twelfth postoperative day.

Discussion

Complications associated with cannulation of the ampulla of Vater and ERCP include elevations of serum and urinary amylase, pancreatitis, ductal rupture, bacteremia, and cholangitis.

Amylase elevation is the most common and most benign of the specific complications. Blackwood (6) found elevated urinary amylase levels in only 6.6 % of patients undergoing gastroduodenoscopy alone, compared with 42.9 % of patients who also had cannulation of the papilla; 3.6 % of the patients had clinical pancreatitis following cannulation of the papilla.

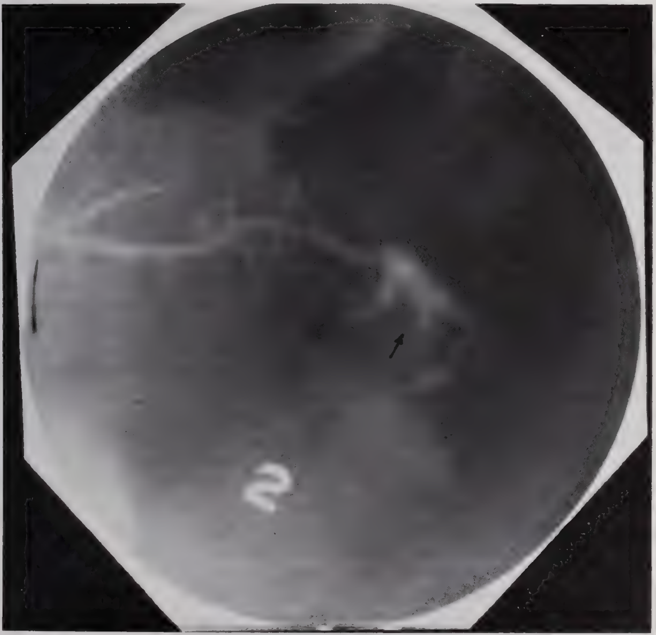

FIG. 3. ERCP which demonstrates irregularity of the distal part of the main pancreatic duct with extravasation (arrow) into the pseudocyst near the tail of the pancreas.

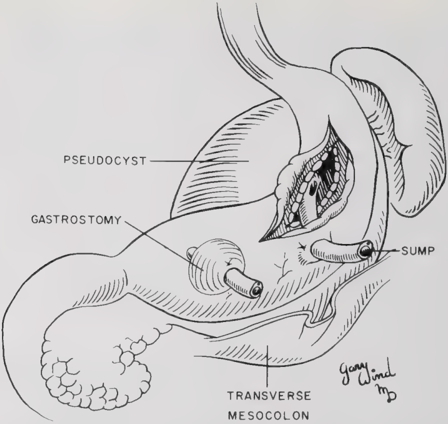

FIG. 4. Pancreatocystgastrostomy and separate tube gastrostomy. Note sump tube placed into the pseudocyst, through the anastomosis, and out the anterior wall of the stomach.

With this risk in mind, the role of retrograde study of the pancreatic duct in suspected pseudocyst must be evaluated. Silvis (7) reported ERCP to be highly reliable in demonstrating abnormal ducts in all 16 patients with confirmed pseudocysts. Although communication between a pseudocyst and a major pancreatic duct has not been thought common, such communication was demonstrated in 12 of these patients. In an additional 13 patients with suspected pseudocyst (10 of whom had an abdominal mass), demonstration of a normal pancreatic duct system by retrograde cannulation made the diagnosis of pseudocyst unlikely and laparotomy was avoided. Silvis (7) also documented resolution of two pseudocysts, although one of these developed transient pancreatitis following the second study. Although the consensus in the literature implies that most pseudocysts require surgical intervention, the experience at our institution has been that many pseudocysts, particularly those following acute pancreatitis, resolve spontaneously. Silvis (7) suggests that endoscopic documentation might aid in selection of such patients.

These benefits must be weighed against the risk of complications. Galvan (8) reported a case of a 35-year-old man who underwent retrograde cannulation for recurrent abdominal pain and elevation of amylase two months after laparotomy for hemorrhagic pancreatitis. Eight hours after the study, he developed severe abdominal pain and fever, and within 48 hours had evidence of a large pseudocyst and pleural effusion. Davis (5) reported 75 patients undergoing ERCP over a 2-year period. In four patients (5.3 %) urgent operative intervention was required to treat septic complications. Two of these had obstructive jaundice, one had pancreatitis, and one had a pseudocyst of the tail of the pancreas. In all cases, stasis could be demonstrated by retention of contrast in the gall bladder or pseudocyst on delayed films. All cases were successfully treated by surgical decompression. The patient with the pseudocyst had onset of abdominal pain and vomiting twelve hours after the study and a fever up to 103°F with positive blood culture, a picture almost identical to the case presented here. A grossly infected cyst containing thick yellow pus was drained.

Several factors have been implicated in an attempt to explain these complications after ERCP. It has been shown that injection of contrast material into the pancreatic duct produces edema, inflammation, and increased permeability of the ductal epithelium (9). It is not clear whether this represents a toxic effect of the contrast material itself, or is secondary to the hyperosmotic effect of the contrast material. It is also well known that several of the commonly used contrast materials cause trypsinogen activation (10) and this could certainly explain the pancreatitis seen after ERCP. Another hypothesis that has been suggested is that excessive pressure during the injection may injure the ductal epithelium. Finally, some authors (8) have suggested that the duct epithelium in patients with previous episodes of pancreatitis may be particularly sensitive to the injection of contrast material.

Several suggestions regarding technique, have been proposed in an effort to reduce the complications after ERCP. These include adequate hydration of the patient, avoidance of pancreatography during an acute attack of pancreatitis, employment of a single injection of a dilute solution of contrast material at a low pressure and aspiration of residual contrast material. Some authors (8) have suggested the use of secretin to flush the duct at the completion of the study. We have found it helpful to institute prophylactic antibiotic therapy in these patients. When there is evidence of duct obstruction, the incidence of septic complications is increased and in these patients antibiotic therapy is mandatory.

Complications following ERCP in patients with pancreatic pseudocysts, have not been reported as occurring any more frequently than in any other group of patients. Silvis (7) reported 16 patients with pseudocysts in whom ERCP was performed. He does not mention the presence or absence of complications.

In our experience with several patients having pancreatic pseudocyst and ERCP, this is the first complication. Although valuable information about the pancreatic duct can be obtained from ERCP, this must be weighed against the possibility of serious septic complications, as occurred in our patient. For this reason, we do not recommend ERCP as a routine procedure in these patients especially when definitive diagnostic information can be obtained by tomography and sonography. When ERCP does demonstrate a pancreatic pseudocyst, we recommend that antibiotics be immediately instituted and that, barring any other contraindications, operative intervention and drainage of the pseudocyst be performed within twenty-four hours.

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Transsphenoidal Microsurgery for Pituitary Tumors: Experience with 45 Cases

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Abstract

The history and technique of transsphenoidal operations for pituitary tumors are reviewed. Fifteen operations were performed for acromegaly, one for Cushing's Disease, and the remaining thirty were nonfunctioning chromophobe adenomas. Tumor size with respect to suprasellar extension did not alter the surgical approach.

Significant and immediate reduction in growth-stimulating hormone levels were obtained in twelve cases out of fourteen. Marked improvement in the visual fields and cranial nerve palsies were obtained in large chromophobe tumors, and reduction in adrenocorticotrophic hormone (ACTH) below normal levels in one patient. Two episodes of meningitis occurred and no reoperations for cerebrospinal fluid rhinorrhea was necessary. There was no postoperative mortality and no worsening of neurologic status.

On the basis of the above results and the comparative mortality and morbidity statistics of transfrontal surgery, transsphenoidal microsurgical removal of pituitary adenomas appears to be the preferred method of therapy in most cases.

Introduction

Tumors in the region of the sella turcica have challenged the ingenuity of surgeons since the turn of the century. The transcranial or more commonly called intracranial approach to tumors of the pituitary was initially attempted. Paul in 1893, Horsley between 1904 and 1906 and Cushing prior to 1910, operated on pituitary tumors by the lateral middle fossa approach (1). At the end of the nineteenth and beginning of the twentieth century, the mortality from the transcranial approach to the pituitary ranged from twenty to eighty percent, but more commonly, sixty to eighty percent, which was the generally accepted mortality of intracranial surgery at that time (2). Giordano, in 1897, may have been the first to suggest a transnasal approach to the pituitary (3). He used a superior nasal incision, entering the sphenoid sinus through the ethmoid cells and nasal cavity. By 1907, Schloffer, an Innsbruck rhinologist (4), successfully removed a tumor by a transnasal approach that involved extensive resection of the turbinates and bony septum, entering the sphenoid through the maxillary and ethmoid sinuses. Within a few years, a number of transsphenoidal operations were performed by various surgeons (4).

In 1909, Kanavel (5) developed a midline, sublabial approach to the pituitary that was soon adopted by Halstead, and later modified by Cushing. Over the next decade, the oronasal procedure, as used by Cushing (2), and the

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endonasal approach developed by Hirsch (6) provided a relatively safe approach for the subtotal removal of pituitary tumors and decompression of the optic nerves and chiasm. In over 300 cases of pituitary neoplasms, operated upon by Cushing and Hirsch, mortality rates of 3 to 4 percent were obtained. Improvement in visual fields varied from 60 to 70 percent comparable to modern craniotomy figures. Cushing diverged from the transsphenoidal route in the late 1920's because of his increasing interest and expertise in intracranial surgery, along with the possibility of obtaining increased exposure and decompression of the optic nerves. In Henderson's analysis of Cushing's statistics (7), he felt that transfrontal operations for pituitary tumors were superior because of increased return of visual acuity and fields to complete normality. Of the total of 60 to 70 percent improving, 20 percent of the transfrontal cases reached a normal, as against only nine percent of transsphenoidal.

During the 1920's, there was a phasing out of the transsphenoidal operation and a gradual transition to the intracranial procedure by most neurosurgeons. However, Hirsch (6, 8), Hamlin (9), and later Dott (7) continued to use the transsphenoidal operation with success. Over the last decade, there has developed renewed interest in the procedure. The introduction of radiofluoroscopic monitoring by Guiot (10) and the dissecting microscope by Hardy (11) represented a significant advance in the technique. The magnification, improved illumination, and fluoroscopic monitoring of instruments within the region of the sella turcica have greatly modified the procedure. The present operation combines the advantages of the original transsphenoidal approach, (eliminates retraction of the brain and the development of seizures, while decreasing the risk of intracranial hematoma), with the increased exposure, visualization, and illumination of the intracranial procedure.

Surgical Technique

Since 1971, we have used Hardy's transsphenoidal technique for practically all operations on the sella turcica. This included the intrasellar portions of two craniopharyngiomas.

The patient is intubated and the pharynx packed with wet gauze. The head is flexed slightly and rotated to the right in a horizontal plane which allows the surgeon access to the space between the shoulder and head and thus brings him closer to the patient. The head is held in a Gardner skull clamp and positioned between the arms of a portable image intensifier with the lateral image of the sella turcica being focused on the TV screen. After the sublabial incision is made, from one canine fossa to the other, the nasal opening of the maxillary bone is rongeured to assume a more rectangular shape. A submucous resection of the inferior one-third of the nasal septum and vomer is carried out and the anterior wall of the sphenoid sinus is opened and removed. With experience, this portion of the operation can be completed in 15 minutes. The Hardy speculum is inserted and retracts the mucosa from the septum and vomer and aids in hemostasis. Using a Guiot spreader the retractor is opened and the turbinates compressed, increasing the diameter of the operative field.

From this point, the operation is performed under the dissecting microscope at 10/16 magnification with especially designed micro-instruments. The sella floor is identified and opened with dissectors when previously eroded by tumor, or a small chisel and fine punches, if the bone is intact. The dissection is confined to the midsaggital plane to avoid the carotid arteries. Bleeding from the circular sinus in the dura can be troublesome and must be controlled by compression with Oxycel cotton or similar hemostatic agents. The larger the intrasellar tumor, the more distortion and compression of the dural sinuses and, therefore, less midline bleeding. The majority of the chromophobe adenomas are semi-solid and occasionally cystic, allowing the bulk of the tumor to be removed by suction. Modified pituitary spoons and ring curettes have been used in removing the residual tumor.

The chromophobe tumor is frequently greyish in contrast with the yellowish color and firm consistency of normal anterior lobe pituitary tissue. In a virgin tumor, easily recognized normal pituitary may be preserved after thorough decompression of the optic nerve and chiasm has been obtained. Frequently, the diaphragm sella is large and the suprasellar cistern herniates into the sella after removal of the bulk of the tumor. In tumors with large anterior suprasellar extension, the herniated cisterns must be displaced superiorly with a small cottonoid and the residual anterior tumor removed piecemeal with curved suctions, pituitary spoons and fine micro-alligator forceps and rongeurs. If a radical tumor removal is being attempted, the dura (tumor capsule) is swabbed with cottonoids dipped in absolute alcohol, to discourage regrowth of tumor cells. When the diaphragm sella is small, and decompression of the cisterns is evident, intraoperative pneumography can be helpful to avoid leaving a large dumbbell-shaped suprasellar portion of the tumor. Occasionally the outline of the optic nerves and chiasm can be visualized through the arachnoid and further confirms adequate decompression of these structures.

The operative procedure is more limited when dealing with a functional adenoma and careful opening of the sella dura allows for the immediate identification of a micro-adenoma that sometimes has a more whitish liquid consistency than typical chromophobe tumors. If a micro-adenoma is present, it is readily apparent, and complete division of the gland is not necessary for exploration. If the change from adenoma to normal pituitary tissue is not clear, it is best to do a subtotal hypophysectomy, utilizing multiple frozen sections for guidance.

After tumor removal, small pieces of previously obtained muscle or subcutaneous fat and fascia lata are used to fill the sella, thus providing hemostasis and preventing herniation of the optic nerve and chiasm inferiorly. A rectangular-shaped piece of septal cartilage is then positioned to create a new sellar floor and prevent emptying of the pack. This closure has prevented cerebrospinal fluid leakage. After removing the Hardy speculum, the nasal mucosa is packed toward the midline with vaseline gauze and the sublabial incision closed with absorbable gut. The vaseline packing is removed in 2 or 3 days, if the sprasellar cisterns are not violated, and 5 or 6 days if the arachnoid space has been opened.

Mortality and Morbidity

There has been no mortality in the series and morbidity was limited to two episodes of meningitis which responded to therapy (Table I). One acromegalic patient had long-standing mild diabetes insipidus controlled by the nasal spray Diapid® (Lysine-8-Vasopressin). Most patients were discharged from the hospital in one week, and there were no reoperations for postoperative bleeding or cerebrospinal fluid rhinorrhea.

Chromophobe Adenomas

Thirty patients with chromophobe tumors underwent surgery. Their ages varied from 16 to 63, with males outnumbering females 20 to 10. Ten patients had previously undergone a full course of radiotherapy, with two patients having started on a second course. Three patients had previous craniotomies with partial tumor removal, done 7, 3 and 1 year prior to the transsphenoidal procedure. Two patients had evidence of pituitary apoplexy and one was blind and stuporous, with total ophthalmoplegia for three days prior to emergency surgery.

Vision

The primary reason for operation in the majority of cases was to improve vision. Twenty-eight patients had visual dysfunction prior to surgery; 24 marked and 4 minimal (Table II). One patient, with normal vision, was a 16-year-old female with secondary amenorrhea and a faint intra- and suprasellar calcification on x-ray. Surgery was advised to eliminate the possibility of craniopharyngioma, which sometimes can be removed while sparing the pituitary gland. Pathologic specimens showed a chromophobe tumor with widespread calcospherite degeneration. The second patient had an aborted pituitary apoplexy and a destroyed sella turcica and was on total hormonal replacement for panhypopituitarism.

Results

Every patient with visual dysfunction showed improvement within 72 hours of the surgical procedure. Several patients with partially cystic tumors had full fields and normal acuity within 48 hours. In 14 out of 28 patients,

Table I

Forty-five pituitary tumors	Morbidity
Rhinorrhea (requiring reoperation)	0
Meningitis	2
Focal neurologic dysfunction	0
Worsening of vision	0
Carotid artery occlusion	0
Cavernous sinus syndrome	0
Permanent diabetes insipidus	1

preoperative visual dysfunction was returned to normal. In virgin cases, visual improvement was usually prompt and satisfactory, while those patients with long-standing marked visual changes and optic atrophy showed more limited gains. Case No. 17 showed immediate improvement but had evidence of decreasing fields and acuity one year later. He refused further pneumoencephalographic investigation. Tumor recurrence was a good probability. The three patients who had extraocular palsies showed marked improvement following surgery.

Endocrine Function

Twenty-five out of thirty patients exhibited some form of hypopituitarism prior to surgery. Gonadal dysfunction was the commonest abnormality and frequently initiated the diagnostic work-up. All patients with severe hypopituitarism preoperatively required hormonal replacement in the postoperative period. Patients with mild hypopituitarism preoperatively and residual pituitary left at surgery, frequently were weaned off cortisone. Almost all the hypogonadal males benefited from testosterone replacement, and 2 of 5 premenopausal females have regained normal menstruation. Even when a radical removal of the sella contents was attempted, the pituitary stalk was infrequently identified due to distortion of anatomy of the diaphragm sella. Residual pituitary tissue attached to the stalk was probably the rule in these cases. Though 50 percent of the patients had some evidence of diabetes insipidus in the immediate postoperative period, there were no patients who required long-term therapy for this disorder.

Radiotherapy

Twelve out of twenty patients who did not have preoperative radiotherapy received it in the postoperative period. Hardy (11), however, omits radiotherapy and follows his patients clinically and radiographically. Though acknowledging his great experience, we feel recurrence should be decreased if the therapy is given. The number of postcraniotomy tumor recurrences was markedly reduced by radiotherapy in large series by Cushing, Henderson (7), Ray and Patterson (12) and the neurosurgical group at the Barnes Hospital in St. Louis (13).

Recurrences

The series is presently too small and the follow-up time too short to provide reliable statistical information. Though most recurrences of chromophobe adenomas occur within the first five years postoperatively (12), they have been reported as late as 13 years later (13).

Functional Adenomas (Acromegaly)

Fourteen patients with acromegaly were operated upon transsphenoidally. There were seven males and seven females with an average age of 47. Most had

Table II

Visual Improvement following Transsphenoidal Excision of Chromophobe Adenomas

Case no.	Tumor extent	Presurgery vision	Postsurgery vision
(1)	Intra- and huge suprasellar asymmetrical L R-intrasphenoid	N.L.P.-O.S. finger count O.D. (R) temporal hemianopsia	N.L.P.-O.S. 20/40-O.D. Full field O.D.
(2)	Intra- and moderate suprasellar	Bitemporal hemianopsia	Normal Vision
(3)	Intra- and moderate suprasellar	Bitemporal hemianopsia 20/80-O.S. 20/40-O.D.	Normal Vision
(4)	Intra- and large suprasellar	20/80-O.S. 20/20-O.D. Bitempora; hemianopsia	20/40-O.S. 20/20-O.D. Residual (L) partial temporal hemianopsia
(5)	Intra- and moderate suprasellar	20/140-O.S. 20/100-O.D. Dense bitemporal hemianopsia Partial 3rd and 6th palsy	20/20-O.S. 20/60-O.D. Minimal bitemporal hemianopsia to color
(6)	Intra- and large suprasellar	Acute ambylopia OU X 3 days complete opthalmoplegia fixed dilated pupils	Light perception and shadows OU. Reactive pupils—Normal E.O.M.
(7)	Intra- and moderate suprasellar	20/40-O.S.-Finger Counting O.D. Dense bitemporal hemianopsia	Normal Vision
(8)	Intra- and marked suprasellar	Dense bitemporal hemianopsia L R	Normal Vision
(9)	Intra- and marked suprasellar	Paracentral scotoma (L). Moderate bitemporal hemianopsia 20/100-O.S. 20/30-O.D.	20/70-O.S. 20/20-O.D. Improvement of inferior quadrants R L
(10)	Intra- and moderate suprasellar	Dense bitemporal hemianopsia	Normal acuity—minimal residual temporal hemianopsia—R L
(11)	Intra- and small suprasellar	Moderate (L) temporal hemianopsia	Normal acuity—Minimal (L) temporal deficit
(12)	Intra- and small suprasellar	Slight bitemporal hemianopsia	Normal Vision
(13)	Intra- and large suprasellar intrasphenoid	N.L.P.-O.S. 20/80-O.D. Dense (R) temporal hemianopsia	Finger counting-O.S. 20/20-O.D. Residual left temporal hemianopsia
(14)	Intra- and large suprasellar	Dense bitemporal hemianopsia	Normal acuity-minimal residual bitemporal hemianopsia

(15)	Intra- and small suprasellar	Minimal (L) temporal hemianopsia	Normal Vision
(16)	Intra- and large suprasellar	20/60-O.S. 20/40-O.D. Dense bitemporal hemianopsia	Normal Vision
(17)	Intra- and large suprasellar	20/150-O.S. 20/100-O.D. Dense bitemporal hemianopsia (L) central scotoma	Initially marked improvement (L) temporal field and acuity. One year post-surgery Vision. O.D.-20/200 O.S. 20/40 Repeat PEG recommended (patient refused)
(18)	Intra- and moderate suprasellar	Normal	Normal
(19)	Intra- and small suprasellar	Minimal bitemporal hemianopsia	Normal
(20)	Intra- and small suprasellar	20/40-O.S. 20/60-O.D. Moderate bitemporal hemianopsia	Normal
(21)	Intra- and large suprasellar	20/200-O.S. finger counting O.D.—Dense bitemporal hemianopsia (R) 3rd nerve palsy	Normal
(22)	Intra- and moderate suprasellar	Moderate bitemporal hemianopsia	Minimal residual bitemporal hemianopsia
(23)	Intra- and small suprasellar	Moderate (L) temporal hemianopsia	Normal
(24)	Intra- and large suprasellar	20/200-O.S. 20/40-O.D. Dense bitemporal hemianopsia	20/80-O.S. 20/40-O.D. Full fields.
(25)	Intra- and large suprasellar	Moderate bitemporal hemianopsia	Normal
(26)	Intrasellar	Normal	Normal
(27)	Intra- and suprasellar intrasphenoid	Bitemporal, superior hemianopsia L R O.S.-20/80	Normal
(28)	Intra- and suprasellar	Bitemporal hemianopsia	Residual inferior Normal Acuity—Temporal defect-O.D.
(29)	Intrasellar and intrasphenoidal	Acuity 20/40-O.D.	Normal
(30)	Intra-suprasellar	Dense bitemporal hemianopsia L R	Minimal (L) peripheral temporal defect
(31)	Intra- and small suprasellar	Normal	Normal

a gradual coarsening of their features and enlargement of hands and feet over several years. A few females presented with typical signs and symptoms of median nerve compression beneath the transverse carpal ligament. Polyarthralgia was another common complaint, while headaches and visual symptoms were uncommon. Endocrine assessment before and after treatment included basal growth hormone levels, glucose and insulin tolerance tests with growth hormone levels, as well as corticosteroid and thyroid evaluation. Preoperative x-ray studies included sella tomography, angiography and pneumoencephalography with coronal and saggital tomography.

Preoperative basal growth hormone levels varied from 6.7 to 1100.0 ng per ml. Normal values are 5 nanograms per ml, occasionally higher with stress, anxiety, fasting or exercise, especially in premenopausal woman (14). Abnormal glucose suppression and insulin stimulation was uniformly demonstrated. Five out of 14 patients had a normal-appearing sella turcica, while the remainder had various degrees of enlargement. Marked distortion of the sella borders was seen less frequently than with nonfunctional adenomas.

Three patients had prior radiotherapy which failed to lower the growth hormone to satisfactory levels. The remaining patients were primary cases. The tumors were macroscropically classified according to Hardy (15): Grade I. microadenoma (normal sella) clear demarcation without capsule between adenohypophysis and tumor (five patients); Grade II. enclosed adenoma with intrasphenoidal and suprasellar expansions (eight patients); Grade III. localized invasive adenoma (dura mater) and bone of the sella floor are destroyed and tumor projects into sphenoid sinus (one patient); and Grade IV. diffuse invasive adenoma (0 patients).

Results (Table III)

In 9 out of 14 patients, postoperative growth hormone levels were reduced to normal range. In two other cases, they were markedly decreased but still somewhat above normal values. In one patient, there was an unsatisfactory decrease from 60 to 39 ng, though initial soft tissue changes occurred. She is presently undergoing a course of radiotherapy. In another, (Case No. 1) a recurrence was noted two years postsurgery with growth hormone levels increasing from 8.8 to 30 and 35 ng per ml. Reoperation and radical removal of sellar contents was done. In Case No. 3, an unusual deep exposure and large asymmetrical sphenoid septum caused technical difficulties which limited exposure and thus tumor removal was unsatisfactory.

Even when growth hormone levels were brought into the normal range, glucose suppression and insulin stimulation tests continued to be abnormal. Soft tissue changes frequently began a few days postoperatively and were complete by one month.. They were more striking in females with a short history and minimal bony changes.

Postoperatively pituitary function was initially maintained in all 14 patients and only the patient who underwent a secondary radical procedure is receiving additional cortisone and thyroid replacement. All patients are being continually evaluated, at six-month intervals.

Table III

Surgical Alteration of Human Growth Hormone

Case no.	Macroscopic classification of adenoma	Type of surgery	Human growth hormone ng/ml	
			Presurgery	Postsurgery
(1)	Enclosed adenoma with small intrasphenoidal expansion	Gross removal of adenoma with pituitary preservation	34.0	8.8—30 + 35 Recurrence 2 yrs. Reoperation 2
(2)	Enclosed adenoma with large intrasphenoidal and small suprasellar expansion	Gross removal of tumor and partial hypophysectomy	1100.0	7.4
(3)	Enclosed adenoma with small intrasphenoidal expansion	Partial gross removal tumor with pituitary preservation—technical difficulties	32—21 Radiotherapy	11
(4)	Enclosed adenoma with small intrasphenoidal expansion	Gross removal of adenoma with pituitary preservation	60.0	39-postsurg Radiotherapy
(5)	Enclosed adenoma with small intrasphenoidal and suprasellar extension	Gross removal of adenoma and partial hypophysectomy	28.0	2.2
(6)	Microadenoma (10 mm in diameter)	Gross removal of adenoma with pituitary preservation	89.0	6.5
(7)	Microadenoma	Gross removal of adenoma with pituitary preservation	6.7	3.4
(8)	Microadenoma	Gross removal of adenoma with pituitary preservation	80.0	6.0
(9)	Enclosed adenoma with small suprasellar expansion	Gross removal of adenoma and subtotal hypophysectomy	42.0	9.3
(10)	Enclosed adenoma with moderate intrasphenoidal and small suprasellar expansion	Gross removal of adenoma and subtotal hypophysectomy	70.0	5.2
(11)	Microadenoma	Gross removal of adenoma with pituitary preservation	40.2	8.2
(12)	Localized invasive adenoma (sphenoidal sinus	Gross removal of adenoma with pituitary preservation	53.5	6.6
(13)	Microadenoma	Gross removal of adenoma with pituitary preservation	24.3	2.3
(14)	Enclosed adenoma with small intrasphenoidal expansion	Gross removal of adenoma with partial hypophysectomy	68.3	5.8

Pathology

Eleven out of 14 tumors were eosinophilic adenomas and the remainder were mixed adenomas.

Cushing's Disease

This patient was originally diagnosed in 1969 and had markedly elevated 17-hydroxycorticoids with significant suppression after Dexamethasone. She refused surgery and received 5000 rads of cobalt irridiation. Marked clinical improvement was noted seven months later; however a progressive rise in 17-hydroxycorticoids and an elevation of adrenocorticotropin levels was noted by 1971.

By April 1975 a full-blown Cushing's syndrome with radiographic evidence of a destroyed sella turcica was found.

At surgery, a radical removal of a mixed chromophobe-basophilic tumor was accomplished. There was an immediate and sustained reduction of 17-hydroxycorticoids, blood pressure, diabetic status and fatiguability. No replacement therapy was necessary.

Comparative Mortality and Morbidity

Postoperative mortality following transfrontal removal of chromophobe adenomas was reported as 1.2 % by Ray and Patterson (12), 4.7 % by Henderson (7) (Cushing's series), 8.6 % by Krayenbuhl, 11.3 % by Olivecrona (16), 14.5 % by Davidoff and Feiring (17), and 10 % by Wirth (13) (Barnes Hospital). Acknowledging the fact that some of the above cases were operated on prior to the advent of synthetic corticoids and osomotic diuretics, hyperventilation anesthesia and other adjuncts to control intracranial pressure, the mortality rate is still significant. Except for the Ray and Patterson report, the mortality rate of transfrontal surgery cannot compare with the transsphenoidal series of Hirsch, Hamlin, Guiot and Hardy, whose mortality rates ranged from a high of 3% to a low approaching zero.

It is noteworthy that the following causes of morbidity following transfrontal surgery are not seen after transsphenoidal surgery: intracranial clots, convulsions, hemiparesis, mental dysfunction, and loss of smell. The worsening of vision following surgery appears to be much less likely to occur after transsphenoidal exposures because the optic nerves are protected by either the dura or the arachnoid, which is not the case in the transfrontal open dura approach to the pitutary.

Radiotherapy of Chromophobe Adenomas

Radiotherapy as the primary treatment might be limited to patients with presumed chromophobe tumors without visual symptoms and with normal pituitary function. Often radiotherapy is recommended to a patient with impaired vision because of the fear of surgical complications. Postradiation visual dysfunction is thought to be acceptable and surgery is not undertaken until marked optic atrophy develops. Recurrence rates of presumed chromophobe tumor following radiation therapy have been reported between 18% and 36% (18, 19) and occasionally a second course of therapy is recommended with limited aspirations and increased possibility of cerebral radiation necrosis. When considering the present mortality and morbidity figures for transsphenoidal microsurgery, patients might be encouraged to accept primary surgical therapy when vision is impaired.

Functional Adenomas (Acromegaly)

The ideal treatment for active acromegaly would seem to be the complete obliteration of the growth-hormone-producing tumor, with preservation of normal pituitary function. On the basis of our series, and Hardy's report of 40 cases, this goal might be realized in about 40 and 50 percent of growth-hormone-producing tumors excised via the transsphenoidal microsurgical approach. We were disappointed to find that abnormal glucose suppression and insulin stimulation persisted despite reduction of growth hormone levels to normal range. Both Hardy and Pearson et al (20) have reported a few cases with normal postoperative glucose suppression values.

Many forms of therapy continue to be recommended for this disorder. They include conventional x-ray treatment (21, 22), proton beam therapy (23, 24) craniotomy with subtotal removal of tumor or complete sellar cleanout; stereotaxic transnasal cryosurgery (25, 26) and stereotaxic implantation of Yittrium-90 (27). None of these methods realizes the above-stated ideal and except for the sellar cleanout, growth hormone levels remain elevated in a substantial number of patients.

We plan to treat recurrence of tumor by radical removal of sellar contents and feel the low morbidity of the procedure warrants initial attempts to leave functioning pituitary tissue at the primary operation when a clear separation seems to exist between tumor and gland.

Corticotropin-Secreting Pituitary Adenomas

Though the presence of gross tumors with enlargement of the sella turcica is rare in Cushing's Disease, the illustrated case typifies the disorder.

When the diagnosis of Cushing's Disease with high (ACTH) levels is established the direct transsphenoidal microsurgical approach might disclose a microadenoma and thus treatment with pituitary preservation as demonstrated by Hardy (28) is possible.

Conclusions

For surgeons experienced both in transfrontal and transsphenoidal microsurgery, the transsphenoidal removal of pituitary adenomas appears to be the preferable method of therapy in the following instances:

1. Visual dysfunction
2. Hormonally active tumor—for selective removal with preservation of pituitary tissue.

3. Midline suprasellar extension of tumor.
4. Intrasphenoidal extension of tumor.
5. Association of CSF rhinorrhea through sphenoid sinus.
6. Pituitary apoplexy.

Contraindications to the transsphenoid route might be:

1. Small sella turcica (with suprasellar extension).
2. Non aeration of sphenoid sinus—3% of population.
3. Extreme asymmetrical lateral extension of tumor.
4. Dumbell-shaped suprasellar extension (diagnosed after transsphenoidal surgery).
5. Uncertainty regarding diagnosis.

Acknowledgment

Endocrinologic evaluation and follow-up was done by Dr. Howard D. Kolodny and staff at the Endocrinology Laboratories of the Queens Hospital Center, Jamaica, New York.

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Myasthenia Gravis and Psychological Illness in an Adolescent: A Case Report

BARRY PERLMAN, M.D., GEORGE HOGBEN, M.D., JESSE ROSENTHAL, M.D. AND
BARBARA WOLF-DORLESTER, PH.D.

Introduction

To our knowledge, no case report of myasthenia gravis and concurrent psychological reactions in an adolescent has been published. This paper describes a 14-year-old girl with myasthenia gravis. She was consciously aware of utilizing her disease as a means of manipulating her social environment. This manipulation was manifest through a misuse of her medications even while under close medical supervision. The case illustrates the multiple interactions among a life-threatening illness, an adolescent girl responsible for taking medications necessary in its control, and one adolescent's need to assert increasing independence while simultaneously requiring continued reliance on parent and doctors for a sense of security.

Case Report

The patient was a fourteen-year-old, single black female with a six-year history of myasthenia gravis. Two weeks prior to this first psychiatric hospitalization she reported to her mother that a surgeon said there was no treatment for a large, "ugly," cheloided thymectomy scar. She became progressively despondent after the consultation and developed anorexia and hypersomnia. She refused to take prescribed anticholinesterase medication, pyridostigmine bromide. She was brought to the hospital by her mother who stated the patient appeared "very upset and nervous."

The patient developed muscle weakness and ptosis at eight years of age. The symptoms were said to have coincided with the father's abandonment of the family. Myasthenia gravis was diagnosed several months after the onset of symptoms and pyridostigmine bromide therapy was instituted. The patient continued in school without academic difficulties. However, she had few friends, never dated, and rarely participated in social activities.

Two years prior to admission the patient began to tire easily. Placement in a special school and home tutorial work were not successful and her education was disrupted. She was hospitalized three times for myasthenic crisis which she provoked by refusing to take medication. During remission from myasthenia, amitriptyline hydrochloride was prescribed for depression. The patient overdosed with amitriptyline following an argument with her mother and was hospitalized on a medical ward. Thymectomy was performed one year prior to the admission reported herein to attempt to arrest her deteriorating medical condition.

The patient was the product of a full-term normal pregnancy and uncomplicated vaginal delivery. She was the fourth born of five female siblings. Developmental milestones were reached earlier than her sisters and she was described as a "fast" child. Menarche at age twelve was described as apparently unremarkable. Her sisters stated she was a quiet shy girl who frequently felt "picked on" by the other family members. There was no family history of myasthenia gravis.

The patient was a tall muscular girl who appeared older than fourteen years old. She had ptosis of both eyelids and no evidence of muscular weakness or muscle wasting in her limbs. She refused

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to speak voluntarily to the staff, except to say that refusing medication was her "weapon against everyone, especially my mother." She acted angry, negativistic, and mildly depressed.

After admission the patient hit a nurse who was in constant companionship to develop a relationship with her. Her companion encouraged her to express her feelings but she refused to verbally communicate or take her medication. Four hours after admission the patient demonstrated extremely shallow respirations and diaphoresis. Preparation for the administration of intravenous pyridostigmine was in progress when spontaneous respiration ceased. Emergency pulmonary resuscitation was successful and unassisted breathing was restored. Gradually, the patient communicated with her companion and accepted oral medication.

The following day she readily socialized, participated in ativities, and took oral medication. However, following an evening phone call with her mother she became upset, tearful, and refused to speak or take medication. After intense staff contact, she began speaking and taking medication. Thereafter, the patient fully cooperated in taking oral medication, communicated freely, and eagerly engaged in activities, including the hospital school.

Psychological testing revealed a full scale IQ of 94 with a Performance IQ of 95 and Verbal IQ of 94. There was no indication of organic impairment. She perceived people as rejecting and related their felt distance to their fears about her illness: "last time I had a friend was last year. Because they knew I was sick they dropped me. . . . They scared, maybe they might get it too." She anticipated ridicule from others that she related to her physical symptoms: "I feel scared, some people stare too much. The way I walk, the way my eyes function, a lot of things." The patient usually received reminders from her mother to take medicine when she refused it. Her earlier suicide attempt had occurred when her mother did not respond to her: "Usually when I don't take my medicine she says something, but this time she said nothing so she was saying she wanted to get rid of me so I took a bottle of Elavil."

Discussion

Chronic disease has an impact on personality development during the teenage years. The adolescent with long-term illness may also use the disease and the medical regime it dictates to manipulate the matrix of social relations. An example of this chronic disease-adolescent interaction has been noted in juvenile onset diabetes mellitus. Rosen and Lidz (1) reported 12 cases of patients whose diabetes mellitus was first diagnosed during their teens and early 20's; in each of these cases there were episodes of acidosis as a consequence of neglect of dietary regimes and omission of essential insulin. Frequently, failures both of diet and insulin regime occurred concomitantly. The authors interpret this behavior as being determined by the patients' desire to manipulate their milieu—"I agree with my mother but go ahead and do as I wish and then blame her if things go wrong." Others may induce hypoglycemia for similar reasons (2).

The same pattern of chronic disease-adolescent interaction might have been anticipated in myasthenia gravis, as it is in many ways a process analogous to diabetes mellitus. The control of myasthenia gravis necessitates the habitual and accurate intake of life-sustaining cholinergic preparations. Life-threatening medical crises can be precipitated by under- or overdosage of prescribed medications. The work reporting the interplay of psychosocial events and this disease has been based on studies of adults. The onset of myasthenia gravis has been linked to points of life stress (3–6). That psychological forces have an impact on the course of the disease and on mortality as a consequence of the disease has been noted (7).

The case presented in this paper is, to our knowledge, the first describing the coexistence of myasthenia gravis and psychologic disturbance in an adolescent. The chronic disease-adolescent interaction in this case of an adolescent with myasthenia gravis does seem similar to those described in diabetic adolescents.

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RADIOLOGIC NOTES

CLAUDE BLOCH, M.D. AND HARVEY M. PECK, M.D., COEDITORS

Cases 388–391

By

BERNARD S. WOLF, M.D. AND HIROSHI NAKAGAWA, M.D.

Case No. 388

A two-and-one-half-year-old female child was admitted because of an ataxic, broad-based, clumsy gait for several months. The child appeared to use her hands awkwardly as well. There was no obvious head enlargement and no evidence of mental retardation or convulsions. General physical examination was negative. The circumference of the head was two standard deviations greater than the average and a cracked-pot (McEwen) sign was present. Papilledema was noted. The cranial nerves were intact. There was hyperspasticity of the extremities, more marked in the lower than in the upper extremities. Diffuse hyperreflexia and bilateral Babinski signs were present.

Plain skull films showed evidence of increased intracranial pressure indicated by separation of the sutures. No evidence of intracranial calcification was present. A computerized head scan (Fig. 1) was performed before and then after injection of water-soluble contrast material intravenously (by infusion of 30% Renografin, 2 ml per Kg). The findings were unchanged after contrast injection. They were interpreted as indicating a large midline subarachnoid cyst in the quadrigeminal cistern and tentorial incisura. The lateral ventricles and the third ventricle were symmetrically dilated without any evidence of deformity or displacement. It was assumed that the cyst was the cause extrinsically of aqueduct obstruction since the fourth ventricle (on the lowermost sections of the scan) was not enlarged although it was displaced downward and backward.

Cerebral angiography confirmed the presence of a space-occupying avascular mass in the region of the quadrigeminal cistern. A right temporo-parietal craniotomy was performed and the right atrium entered. The medial wall of the right atrium was then punctured and a cyst containing clear yellowish fluid was aspirated. The fluid clotted on standing. A teflon multiple perforated flanged catheter was shortened and the flanged end inserted into the cyst from the atrium and left in situ in order to establish free communication between the cyst and the ventricular system. The postoperative course was complicated by a subdural and subgaleal fluid collection which required a subdural-peritoneal shunt. After this, the child did very well. Repeat computerized

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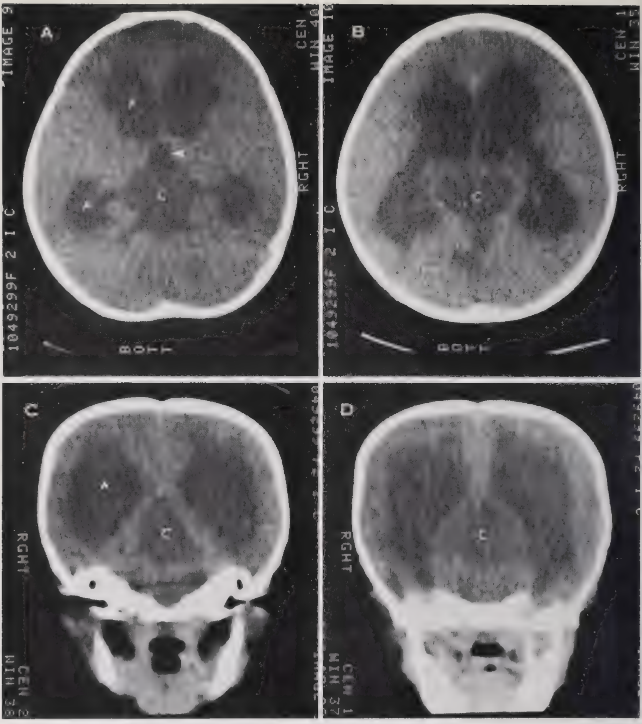


Case 388, Fig. 1. Computerized head scan.

Case 388, Fig. 1A. Transverse axial section parallel to the supraorbital-meatal plane through the frontal horns anteriorly and the atria posteriorly shows markedly dilated symmetrical frontal horns (F) and atria (A). The right side of the patient is on the viewer's right. The anterior portion of the third ventricle (arrowhead) is dilated but in the midline. A most unusual finding is a large midline lucent pentagonal shaped area (C) with an attenuation number slightly higher than cerebrospinal fluid (8 to 10 on the Delta number scale). This fluid-filled area occupies the quadrigeminal cistern and extends into the cisterna velum interpositum.

Case 388, Fig. 1B. Transverse axial section just above the section shown in figure 1A shows a more triangular configuration to the midline cystic structure (C) located between the relatively compressed mid-portions of the bodies of the lateral ventricles.

Case 388, Fig. 1C. A coronal or frontal section through the external auditory canals; the right side of the patient is on the viewer's left. This "antero-posterior" section shows that the cystic lesion (C) is precisely in the midline and fits neatly between and below the markedly dilated atria (A).

Case 388, Fig. 1D. A coronal section anterior to figure 1C shows the lateral contours of the cystic structure (C) to be more convex laterally at this level and to bulge against the medial walls of the dilated lateral ventricles.

scans have shown diminution in the size of the cyst and of the ventricles. Papilledema disappeared and on discharge the child was walking normally.

Acknowledgment

The authors wish to thank Dr. Charles B. Brill and Dr. Leonard I. Malis for permission to publish this case.

Case Report: SUBARACHNOID CYST OF THE QUADRIGEMINAL CISTERN.

Case No. 389

A girl of seven was ambulatory when referred for a computerized head scan. In early infancy, the parents noted unusual enlargement of the head. Physical and neurologic findings at that time were noncontributory although transillumination of the posterior fossa was positive. An angiogram done at the age of three was interpreted as possible Dandy-Walker syndrome and agenesis of the corpus callosum. The patient was treated with Diamox quite successfully because the hydrocephalus appeared to be arrested. Clinically the child was doing well except for a borderline mental status. A computerized scan (Fig. 1) was consistent with the original diagnosis of the Dandy-Walker syndrome and agenesis of the corpus callosum.

Acknowledgment

We wish to thank Dr. Martin R. Geller for permission to publish this case.

Case Report: PROBABLE DANDY-WALKER SYNDROME AND AGENESIS OF THE CORPUS CALLOSUM.

Case No. 390

A 68-year-old man was referred because of a history of memory loss and difficulty in walking of several months' duration. Neurologic examination confirmed a mild organic mental syndrome, poor coordination and a broad-based gait, but no focal or localizing signs. Electroencephalogram showed diffuse abnormalities bilaterally. An isotope brain scan and skull films were negative. A computerized head scan was requested and the findings (Fig. 1) were surprising because the changes were typical of agenesis of the corpus callosum which apparently had been asymptomatic all his life. There was in addition a fairly marked degree of enlargement of the cortical sulci symmetrically over both hemispheres and ventricular dilatation consistent with the organic mental syndrome that the patient had developed several months before.

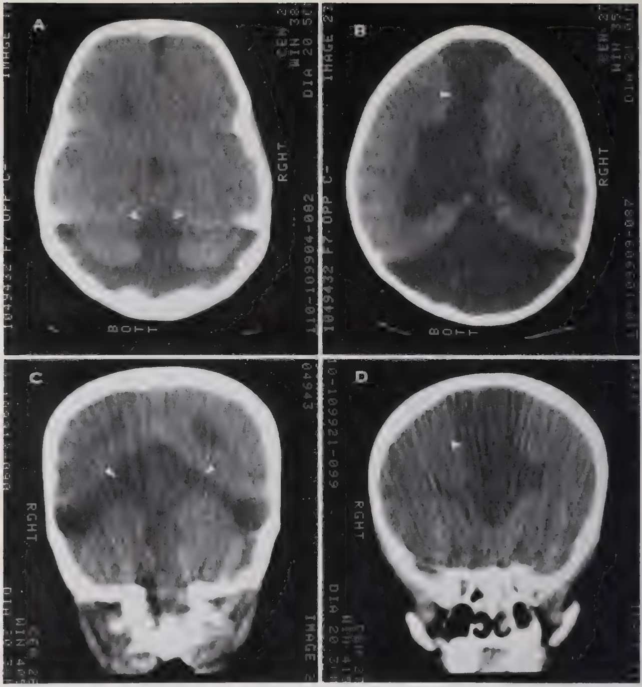
Acknowledgment

We wish to thank Dr. Edward J. Reich for permission to publish this case.

Case Report: AGENESIS OF THE CORPUS CALLOSUM IN A 68-YEAR-OLD MAN.

Case No. 391

A five-month-old male infant was admitted because of psychomotor retardation and an asymmetrical head. He was known to have arthrogryposis,



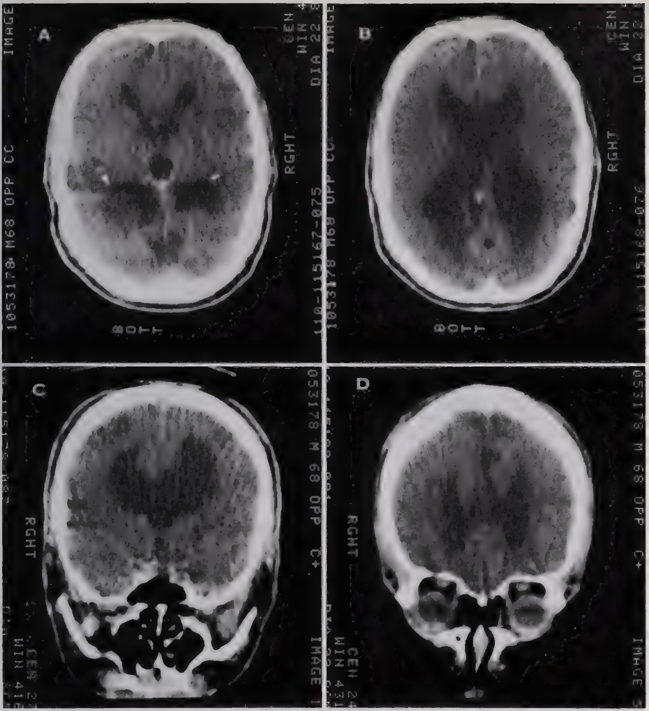
Case 389, Fig. 1. Computerized tomography without contrast injection.

Case 389, Fig. 1A. A transverse axial section parallel to the supraorbital-meatal plane below the level of the frontal horns anteriorly and through the region of the fourth ventricle (arrowheads) posteriorly. The ambient and interpeduncular cisterns outline the mesencephalon. The region of the fourth ventricle communicates through a wide channel with a lucent fluid-filled area between and behind the cerebellar hemispheres.

Case 389, Fig. 1B. A transverse axial section above figure 1A shows that the upper portion of the posterior fossa is completely lucent with a triangular configuration, apex located anteriorly in the midline. The posterior fossa extends unusually high since the petrous ridges are not seen in the section. The anterior portion of the interhemispheric fissure (arrowhead) is markedly widened and appears to communicate directly with a dilated irregularly shaped third and left lateral ventricle. The right lateral ventricle is poorly developed.

Case 389, Fig. 1C. A coronal or frontal section through the hugh posterior fossa shows the markedly elevated tentorium (arrowheads) outlined by a wide lucent area beneath it. Extending downward from this area in the midline, there is a broad communication with the region of the fourth ventricle.

Case 389, Fig. 1D. A coronal section anterior to figure 1C confirms the markedly dilated interhemispheric fissure (arrowhead) which communicates inferiorly with an elevated dilated third ventricle and deformed left lateral ventricle. The right lateral ventricle is small with a bizarre shape.



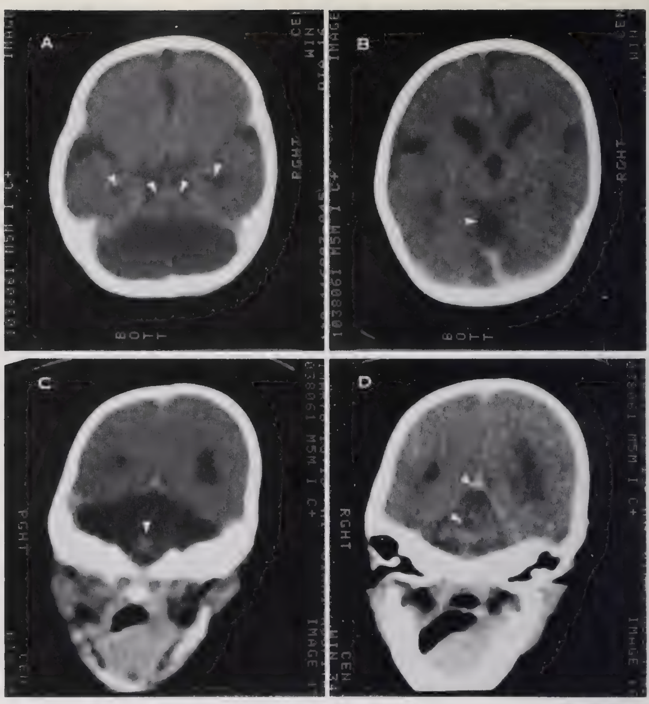
Case 390, Fig. 1. Computerized scan after contrast injection.

Case 390, Fig. 1A. Transverse axial tomogram parallel to the supraorbital-meatal line through the frontal horns anteriorly and the atria posteriorly. The atria (arrowheads) are disproportionately large and extend almost to the midline indicating an absence of the splenium of the corpus callosum. The third ventricle is dilated and elevated. The posterior portions of the internal cerebral veins outline the posterior part of the third ventricle.

Case 390, Fig. 1B. Transverse axial section at a higher level than Figure 1A shows markedly dilated lateral ventricles with pointed frontal horns and markedly dilated elevated atria located unusually close to each other and to the midline.

Case 390, Fig. 1C. A coronal or frontal section through the sella turcica shows a "bat-" or "butterfly-shaped" configuration of the atria and posterior horns with elevation of the third ventricle.

Case 390, Fig. 1D. A coronal section anterior to Figure 1C again shows the curious winged configuration of the lateral ventricles and the elevated third ventricle. The interhemispheric fissure is dilated and widened cortical sulci are seen over the convexities.



Case 391, Fig. 1. Computerized tomography after contrast injection.

Case 391, Fig. 1A. Transverse axial section a short distance above the sphenoidal and petrous ridges shows almost complete lucency of the posterior fossa except for the region of the brain stem (medial pair of arrowheads) behind the basal cistern. The tips of the temporal horns (lateral pair of arrowheads) are dilated. The Sylvian and the anterior interhemispheric fissures are widened.

Case 391, Fig. 1B. Transverse axial section above Figure 1A shows dilatation of the supravermian cistern (arrowhead) anterior to the posterior portion of the opacified falx cerebri. The ambient cisterns, dilated midline third ventricle, dilated frontal horns and dilated Sylvian and anterior interhemispheric fissures are also seen. There is no evidence of displacement or any focal mass lesion.

Case 391, Fig. 1C. Coronal or frontal section through the posterior fossa again shows it to be practically filled with fluid except for the brain stem (arrowhead). The tentorium is elevated.

Case 391, Fig. 1D. Coronal section anterior to Figure 1C shows the large supravermian cistern below the apex of the tentorial notch (upper arrowhead). A small midline somewhat irregular lucency (lower arrowhead) is in the expected region of the fourth ventricle. Portions of the lateral ventricles are also seen. Other sections demonstrated a moderate degree of dilatation of the lateral ventricles which were symmetrical except for a relatively small right posterior horn. The third ventricle is rather markedly dilated but not displaced or deformed.

most marked in the lower extremities which had required treatment with casts. The parents had noted that he was unable to lift his head, did not smile or respond promptly to stimuli. Neurologic examination showed that the child demonstrated little spontaneous movement, a weak sucking reflex, generalized hypotonicity, and a poor response to painful stimuli. The head circumference was within normal limits. The cranial nerves were intact.

A computerized tomogram of the head after contrast intravenous injection (Fig. 1) was performed and showed an extraordinarily lucent posterior fossa due to absent or markedly compressed and atrophic cerebellar hemispheres. The third and lateral ventricles were dilated. No further investigation was performed.

Case Report: AGENESIS OF THE CEREBELLUM OR POSTERIOR FOSSA CYST [SUBARACHNOID OR DANDY-WALKER SYNDROME] WITH HYPOPLASIA OF THE CEREBELLUM.

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PEDIATRIC NOTES

AARON R. RAUSEN, M.D., EDITOR

The Return of the Displaced Kidney: A Diagnostic Dilemma during Therapy of a Retroperitoneal Sarcoma

By

DUKE O. KASPRISIN, M.D. AND AARON R. RAUSEN, M.D.

The evaluation of new masses in children being treated for documented malignancies usually denotes recurrent disease even when the original neoplasm appears to have been effectively treated. The present case report presents the appearance of a new mass in a patient with known retroperitoneal sarcoma who had received radiotherapy, chemotherapy, and surgery. Diagnostic studies revealed the "new" mass to be a kidney previously displaced by tumor that was undergoing repositioning as the displacing tumor regressed.

Case Report

T. D., a white female, was first admitted to another hospital at 27 months of age, with a one-month history of anorexia, weight loss, and abdominal protuberance due to an upper abdominal mass. An intravenous pyelogram and an upper gastrointestinal series verified that the mass was retroperitoneal. The left kidney was displaced infero-medially to the level of L4-L5 and the stomach and small intestine to the right side of the abdomen. Prior to surgery, the tumor mass was irradiated with 800 rads. At laparotomy, the retroperitoneal mass was inoperable, extending across the midline into the liver, with left diaphragmatic implants. Histologic section was interpreted as showing a primitive sarcoma compatible with rhabdomyosarcoma. There was no evidence of distant metastasis. Two days after surgery, she was given a five-day course of actinomycin D and two injections of cyclophosphamide. When the prognosis was deemed hopeless and chemotherapy was discontinued, further consultation was sought by the family and the child was admitted to the Beth Israel Medical Center at 29 months of age.

A repeat intravenous pyelogram showed the mass to be of the same gross size and location as in the preoperative period (Fig. 1). The child was started on combination therapy with intravenous vincristine, adriamycin, cyclophosphamide, and actinomycin D. The tumor mass shrank dramatically so that it was only questionably palpable after 8 weeks of therapy. A "second look"

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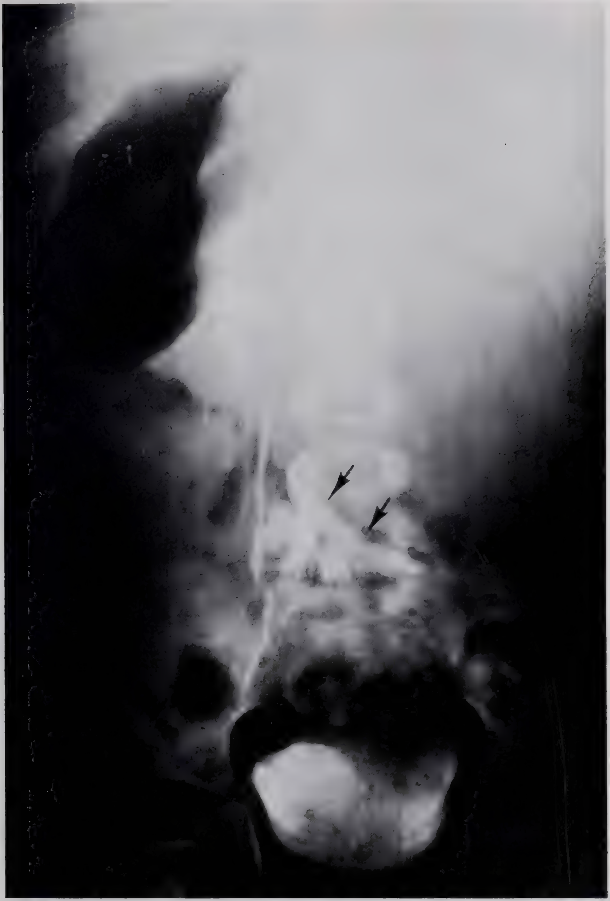

FIG. 1. Intravenous pyelogram at 29 months of age demonstrates a left abdominal mass displacing and rotating the left kidney inferiorly and to the right. The stomach is shifted across the midline.

laparotomy was performed at 32 months to assess tumor size and operability. At surgery, the tumor mass was remarkably smaller but still involved most of the left upper retroperitoneal area. The left kidney was now displaced downward only to the midleft abdomen. The tumor still extended to the diaphragm superiorly and involved by contiguity the left lobe of the liver and the spleen. These residual masses were still considered inoperable and the outer borders were marked with radioopaque clips. Histologic examination of multiple biopsies of the mass revealed only granulation tissue and no evidence of malignant cells. Beginning 13 days postoperatively, the area demarcated by the clips received 5000 rads over a 6½-week period.

The child did well being continued on combination chemotherapy with cyclophosphamide, actinomycin D, vincristine, and adriamycin.

At 36 months of age, an ill-defined left flank mass was felt. Roentgenograms showed the prior shrunken mass under the left hemidiaphragm as well as a

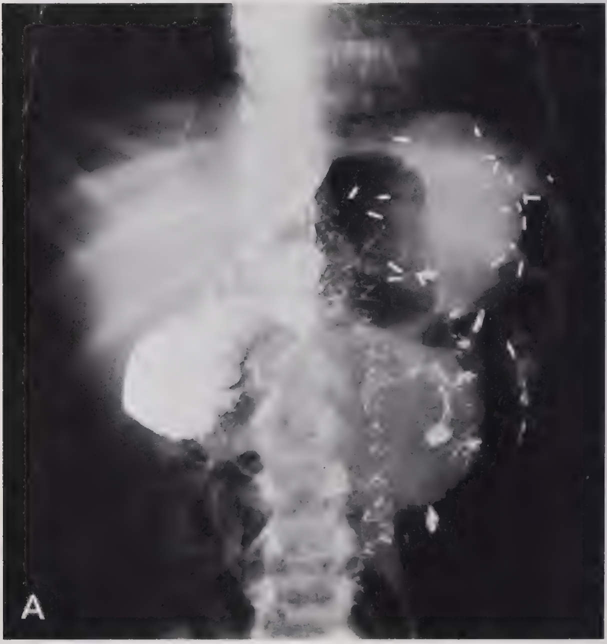

FIG. 2a. Eight months after initial exploration, an abdominal x-ray following a GI series reveals a left upper quadrant mass as well as a palpable left midquadrant mass adjacent to the spine.

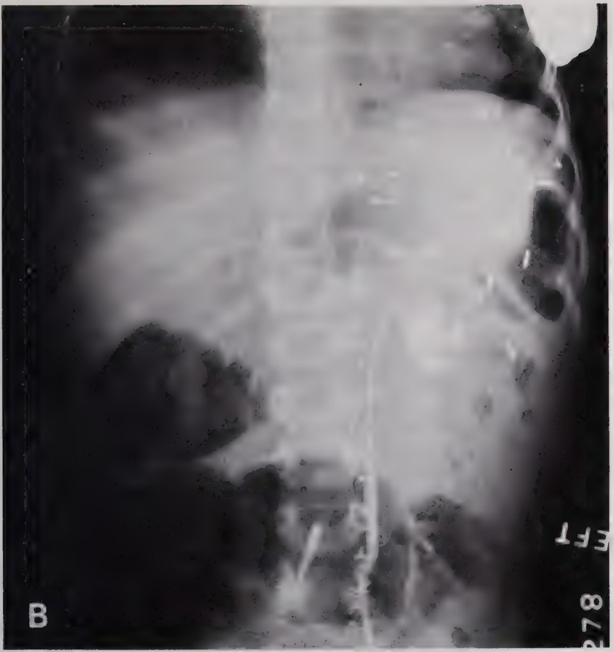

FIG. 2b. Left retrograde pyelogram demonstrates that the left midquadrant mass is the left kidney which has ascended to this position after therapy.

"new" mass in the left midabdominal paravertebral area. The new mass appeared to be encroaching into the left colon medially but could not be defined optimally on barium enema and upper gastrointestinal series (Fig. 2a). Since the child had had a cutaneous allergic reaction following a prior intravenous pyelogram, a left retrograde pyelogram was performed to reevaluate the position of the left kidney and the mass. The mass was shown to correspond exactly to the position of the left kidney (Fig. 2b).

At 43 months of age, she was readmitted to the hospital for fever, vomiting, left upper quadrant abdominal pain and constipation. A barium enema revealed a complete obstruction at the splenic flexure. At laparotomy, tumor was found encasing the descending colon and penetrating through the wall of the bowel. Resection of the splenic flexure and a transverse colostomy were performed. Her postoperative course was associated with a Bacteroides ovatus septicemia which responded well to parenteral antibiotics. The local tumor remained stable for 2 months utilizing combination chemotherapy with intravenous cycles every three weeks of vincristine, cyclophosphamide and dimethyltriazenoimidazole carboxamide.

When left pleural effusion occurred she was changed to MOPP chemotherapy at 46 months of age. The left upper quadrant mass continued to enlarge and she again developed complete intestinal obstruction requiring a jejunojejunostomy to bypass the tumor obstruction. Her condition continued to deteriorate and she died at 48 months of age.

At autopsy, an undifferentiated retroperitoneal sarcoma was documented, invading the left hemidiaphragm, left lung, spleen, left adrenal gland and kidney, and the large and small intestines.

Discussion

In the human embryo, the kidney is formed in the caudal portion of the abdomen. It moves cephalad associated with the rapid growth of the caudal portion of the body. However, the kidney itself also migrates cephalad by some other mechanism (1). In the newborn period, if the kidney is displaced and twisted by adrenal hemorrhage, it will return to its normal position upon resolution of the hemorrhage (2).

In this case report, a retroperitoneal sarcoma was found pushing the kidney downward and medially. When the tumor temporarily regressed with combination radiotherapy and chemotherapy, the kidney again moved cephalad toward its "normal" position.

When the kidney is displaced by any suprarenal mass, there may be a mechanism analogous to that which operates in the embryo which will return the kidney to its normal position once the displacing force has been removed. Thus during the migration of a displaced kidney back toward its normal position, if the kidney becomes palpable in an aberrant position where tumor had been prior to effective therapy, a diagnostic dilemma may occur.

Summary

This paper describes a 2-year-old child who had a massive retroperitoneal sarcoma displacing the left kidney downward and medially. After combination chemotherapy and radiotherapy, the kidney became a palpable mass in moving toward its normal position. The diagnostic dilemma of tumor versus kidney was resolved by contrast studies.

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Student Section

Peptic Ulcer Disease and Gastric Hypersecretion following Portacaval Shunt

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For the past 30 years, one of the primary methods of treatment for the management of portal hypertension has been the creation of a portal-systemic anastomosis. Among the alleged complications of such an operation has been an increased incidence of encephalopathy, hemosiderosis, and peptic ulcer disease. It is generally accepted that there is an increased incidence of the first two complications mentioned. However, it has been a source of continuing controversy for many years as to whether there is an increase in the incidence or severity of peptic ulcer disease following a portacaval shunt (PCS). This paper will discuss whether or not there is an increased incidence, as well as why such an increase might occur.

The first PCS was performed in 1877 in dogs by Nikolai Eck; the result subsequently became known as an Eck's fistula. He thought it could be used in humans for the treatment of ascites (1), but early attempts were unsuccessful. In 1933, Lebedinskaja noted that there was an increase in the gastric secretions of dogs who had Eck's fistulas (2). This was confirmed in 1937 by Gerez and Weiss (3), and they were the first to propose that perhaps some portal blood secretagogue was bypassing the liver, escaping inactivation, and stimulating gastric secretions.

This early work was ignored until 1957, when Dubuque et al reported that 15% of 60 patients with PCS developed a peptic ulcer after 8 years. They constructed Heidenhain pouches in dogs to study this phenomenon. They divided the dogs into 3 groups. One group had no other procedures, a second had a PCS constructed, and a third had a PCS constructed one month after ligation of their portal vein, to create portal hypertension, in the hope of mimicking the human situation. They found no increase in acid output in the first group, but a 300% increase in total acid output in the second and third groups. They then gave maximal histamine stimulation to all three groups. The two groups with PCS developed a higher incidence of peptic ulcers, and died sooner of perforations than the control group. They concluded that some gastric secretagogue was escaping hepatic inactivation, and that this secretagogue was probably histamine (4).

These results were not immediately accepted, because one year later Clarke et al reported that they had studied gastric secretion in patients with cirrhosis

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and portal hypertension and compared them to patients with PCS. They found no significant increase in acid output in those patients with PCS, either in basal secretions or after a meal (5). In following 62 patients with PCS for three years, only four developed ulcers, and all of these occurred within 10 months of the operation. They therefore felt that these were stress ulcers and were not solely a result of the PCS. They also repeated the studies in dogs with Heidenhain pouches and found a great increase in acid output following PCS. Neither a vagotomy nor an antrectomy affected this increase, so it seemed that the secretagogue was some humoral factor other than gastrin. However, the rise in acid output occurred in correspondence with meals, so they thought the humoral factor was released into the portal vein following a meal and escaped hepatic inactivation. They stressed that the results between normal dogs and cirrhotic patients could not be directly compared (6).

That the secretagogue was not gastrin was confirmed by Kohatsu et al (7). They isolated Heidenhain pouches in dogs under the skin, performed a vagotomy, antrectomy, and esophagoduodenostomy. They still got a 537% increase in the free acid output/24 hours, comparing the same dogs before and after PCS construction. Because of the time course between food ingestion and acid output, they thought the secretagogue probably came from the duodenum. Recently, gastrin was definitely ruled out as the secretagogue following the development of a radioimmunoassay for it. Clendinner et al found a decrease in fasting and postprandial serum gastrin levels in dogs following PCS. They also showed that there is normally no gastrin metabolism in the liver (8).

Silen and Eiseman fed dogs with PCS the various components of food separately and found that only the protein component caused gastric hypersecretion. Then they performed a splenectomy and pancreatectomy on the dogs, and neither of these operations abolished the hypersecretion. They concluded that a secretagogue coming from the intestine responds to the proteins in food, escapes hepatic inactivation and causes the stomach to secrete acid. They thought the substance was histamine because histamine levels normally rise markedly following protein ingestion, whereas other dietary components do not cause this rise. Also, histamine is normally metabolized in the liver (9). Support for histamine came from the experiments of Irvine et al. They first showed that histidine within ingested meat is decarboxylated by intestinal bacteria to histamine, resulting in increased absorption by the intestine (10). They further showed that this increased absorption gives rise to both an increase in total urinary histamine and gastric acid output following PCS. The same results were obtained in normal dogs with administration of amino-guanidine, an inhibitor of hepatic histaminase (11). They felt that this explained Clarke's work with dogs (6).

Clarke et al went on to try to determine the site of origin of this secretagogue. He constructed a mesocaval shunt in dogs, so that blood from the jejunum, the ileum, and colon bypassed the liver, whereas the blood from the stomach, spleen, pancreas, and duodenum did not. They still found a large increase in gastric acid output following PCS. When blood from the small and large intestine was redirected into the liver, this increase disappeared. They concluded that the secretagogue came from the intestine at a point distal to the duodenum (12).

Two years later, in 1961, Silen and Eiseman came out with what they thought was further evidence for histamine being the secretagogue, but in retrospect they reported the first results against it. They found a significant increase in the arterial blood concentration of histamine following a protein meal in dogs with PCS. In normal dogs, they found a significant increase in histamine concentration in the portal vein over the hepatic vein following a protein meal. They thought that this proved that histamine avoided hepatic metabolism and caused gastric hypersecretion. However, they did not find a significant increase in arterial blood histamine in dogs with PCS compared to normal dogs. Also, the blood histamine levels did not rise for 2 to 4 hours following the meal, while the rise in acid output occurred immediately (13). So it was not clear if histamine was the secretagogue in dogs, and, more importantly, it was not clear if any of this related to humans with PCS, and if this caused them to develop peptic ulcers.

Ostrow et al (14) repeated Clarke's (5) work in humans. They found a significant increase in gastric secretions in eleven cirrhotics with PCS compared to 29 cirrhotics without. However, they were not matched for age or degree of cirrhosis. Also, the increase in acid output is nowhere near as great as that found in dogs following PCS. They explain this by saying that cirrhotics already have portal hypertension, and because of this, develop marked portosystemic collaterals before the PCS is constructed. Therefore, the PCS may not significantly affect the amount of secretagogue reaching the stomach, but only relieve the portal hypertension. This is not supported by Dubuque's original finding (4) of an increase in acid output in dogs whose portal vein had been ligated before the PCS was constructed.

Bendett et al (15) studied the gastric secretion in eleven cirrhotic patients before and after they had a PCS. He utilized the augmented histamine test as well as intragastrically administered histamine and measured acid output. He found no significant increase in acid output immediately after the PCS or six months later. They felt their work was more conclusive than Ostrow's (14) because they used each patient as his own control and because they used large doses of histamine to stimulate acid output. They stated that PCS does not increase gastric secretions in humans, and that the increase in dogs occurs because they do not have portal hypertension or any of the other physiologic changes that occur in cirrhosis.

During the 1960's many centers issued reports on their experience with PCS in large series of patients, and there were conflicting results as to whether this operation caused an increased incidence of peptic ulcer disease. In 237 patients at Mass. General, McDermott et al (16) found a 15% incidence of peptic ulcers in patients who had undergone PCS. In 131 patients at N. Y. Hospital, Warty and Payne (17) did not find a greater percentage of ulcers in their patients with PCS than in reported series of cirrhotic patients who did not have PCS (around 5%). In those patients who did get ulcers, however, the incidence of severe symptoms was very high. In 230 patients, Mikkelson et al (18) reported only a 7% incidence of peptic ulcers. More than half of these died from their ulcers, and they also felt that the virulence of these ulcers was greater. Rousselot et al (19) reported a 6% incidence of ulcers, and half of these were recurrences. Liebowitz (20) studied autopsies of patients with PCS and found a 16% incidence of peptic ulcer disease. All of this occurred within 10 months of the operation so he felt that they were temporally related. Wilkinson and Riddel (21) in England reported a 10% incidence of peptic ulcers following the shunt. However, none of these patients had an increase in gastric acid output following a maximal stimulating dose of histamine. It is difficult to draw conclusions from any of this work because all of them are nonrandomized, retrospective, uncontrolled studies.

In an attempt to more closely relate the findings in experimental animals to the human situation, Hein et al (22) produced hepatocellular damage and cirrhosis in dogs by CCl_4 administration. They then measured acid secretions after constructing a Heidenhain pouch in each animal, and found it to be increased. The acid secretions increased even further after a PCS was constructed. Since this is the opposite of what Bendett (15) found in humans, the relevance of all the work in animals was brought into question.

Nevertheless, the phenomenon of gastric hypersecretion in dogs following PCS was looked on by physiologists as an important aspect in understanding the control of gastric secretions, and much work was done to elucidate the nature of the intestinal hormone which caused it. Rex et al (23) repeated the work of Irvine (10) with a more precise assay for the measurement of urinary histamine. They found no increase in the amount of urinary free histamine following the ingestion of a meat meal in dogs with a PCS. Further evidence that histamine was not the secretagogue came from Windsor et al (24). First they measured the gastric secretions and arterial blood histamine levels of dogs with Heidenhain pouches. They then ligated the hepatic veins of these dogs and repeated the measurements after the formation of ascites, portal hypertension, and histologic evidence of liver damage on liver biopsy. They found a significant decrease in the gastric secretory response to all stimuli in every dog. They then formed a PCS in the dogs and every dog developed a marked gastric hypersecretion. However, there was no significant difference in the arterial blood histamine levels following a meal between the normal, ascitic, or shunted dogs. They concluded that the gastric hypersecretion was not due to histamine.

This was confirmed by the study of Newman et al (25). They measured the histidine decarboxylase activity of the gastric mucosa before and after PCS. They found no significant change in the activity of this enzyme. In addition, they could not inhibit the gastric hypersecretion following PCS with the administration of brocresine, an inhibitor of histidine decarboxylase.

More evidence against histamine was reported by Bader et al (26). They collected the urine from 20 cirrhotic patients before and after they had a PCS. They then injected the urine into rats and measured the gastric secretions of the rats. There was not a significant increase in the acid output of the rats following the administration of the post-PCS urine. This study is not conclusive, however, because rats respond only to very high concentrations of histamine, while the histamine concentration in the urine was low. Finally, Griffen et al (27) measured the rate of rise of blood histamine levels and compared it to the rate of rise of gastric acid output following PCS in dogs. They found no correlation between the two. The only substance that rose at the same rate as acid output was blood ammonia.

The most definitive work in elucidating the nature of the secretagogue came from Orloff and his group (28–36). First they proved that there is a humoral gastric secretagogue released into the portal circulation following the entrance of food into the intestine in the following way. In 14 dogs they constructed Heidenhain pouches and performed an antrectomy and Billroth II gastrojejunostomy. They gave the dogs a meal through an indwelling catheter placed in the duodenum and measured gastric acid output. They then placed catheters in the portal vein and thoracic aorta of the dogs, and following the meal, removed blood from the portal vein and put it into the aorta at timed intervals. They found that the control meal had no effect on gastric acid output. Following the blood exchange, the acid output increased in a dose-response related fashion to the amount of blood exchanged (28).

To confirm this, they performed a similar experiment utilizing cross-transfusion between two different dogs (29). They took blood from the portal vein of dogs who had a PCS before and after they received a meal through a jejunal fistula, and injected it into dogs with an intact hepatic circulation. There was no rise in acid secretion in recipient dogs when injected with blood from controls, but there was a 244% increase in acid output when recipients received portal blood from shunted dogs. In addition, they placed some radioactive serum albumin in the food given to the shunted dogs. There was no correlation between the rate of absorption of the albumin and the rise in acid output. Thus, they had direct evidence for a humoral stimulant of gastric secretion that escaped liver degradation in shunted dogs. The results also suggested that this hormone was of endogenous origin.

They went on to see if what they called the intestinal phase of gastric secretion occurred in man (30). They instilled food through a nasointestinal tube placed in the jejunum in 10 normal adults, 20 compensated cirrhotics, and 15 compensated shunted cirrhotics. They measured the amount of gastric secretions through a nasogastric tube. Normals had a 20% decrease in gastric secretions, and cirrhotics had a 31% decrease. Shunted cirrhotics, however, had a 707% increase in gastric secretions. This proved that the creation of a PCS unmasked this phase of gastric secretions by allowing some hormone to reach the stomach.

Next they found exactly where the hormone was produced. First in dogs with PCS (31) they instilled separate meals into the jejunum, ileum, and colon. They found a significant increase in gastric secretions only following the jejunal meal. They repeated this in patients with PCS and got the same result (32). In cirrhotics without PCS, no meal produced such an increase. Therefore, the hormone originates in the jejunum.

They then showed that the hormone was endogenous and not absorbed from food. They again fed dogs with a PCS through a catheter in the jejunum and compared the increase in gastric secretions to that produced when a balloon attached to the catheter distended the jejunum. They got a very similar increase in secretions from either the food or the balloon distension (33). The study was then repeated in man. Normals and unshunted cirrhotics responded to neither the jejunal food nor the balloon distension, while shunted cirrhotics responded to both with an almost 800% increase in the volume of gastric secretions (34).

Orloff's group was then able to isolate a crude extract that contained this hormone (35). They assumed the hormone to be a low molecular weight polypeptide similar to other GI hormones and used techniques similar to those used in the isolation of gastrin, CCK-PZ, and secretin. The isolated extract came from the intestinal mucosa of a pig and increased dog gastric acid secretion in a dose response relationship. They were also able to show that this hormone worked through the cyclic AMP system in the gastric mucosa (36). There was a dose response relationship between the extract and cAMP levels in gastric mucosa. The purification and exact nature of this hormone has not yet been reported.

Although the existence of such a hormone in man was known by 1969, it was not yet known if its presence led to an increased incidence of peptic ulcer disease following PCS. At this time, the results of controlled, randomized, prospective studies on patients with PCS began being published, so that this question could be answered definitively.

Orloff and his group reported a prospective study on 92 alcoholics (30). Sixty of the patients were shunted (40 as emergencies), and 32 were treated medically. The patients were not randomized. The patients were of similar age, and had similar degrees of cirrhosis. Four of the medical patients developed ulcers (12%) and 16 of the shunted patients did (27%). However, 14 of these 16 ulcers were superficial erosions of the gastric mucosa that occurred less than 30 days after the operation (stress ulcers). Since most of the shunts were done as emergencies, one might expect a high incidence of stress ulcers in this group. Therefore this study is not conclusive.

Jackson et al studied randomized patients and analyzed both the prophylactic PCS and the therapeutic PCS. He found a significant increase in the amount of peptic ulcers seen at autopsy in those patients who underwent prophylactic PCS vs. those treated medically (13.5% vs 3.4%) (37). There was no significant difference in those who received the therapeutic operation (for bleeding varices) versus the medical group (38). However, none of the patients were matched for factors such as age or degree of cirrhosis.

Resnick et al (39) did a controlled study of the prophylactic PCS and did not find a significant increase in peptic ulcers. However, they were more interested in other data about the operation and peptic ulcers were only looked for incidentally. Conn (40) performed a controlled prospective study on shunted and unshunted cirrhotics that were matched for age and severity of disease. He found no significant increase in peptic ulcers in the shunted group. His patients, though, were not randomly selected and the control group was followed for a shorter period of time.

A well-planned, definitive study was finally reported this year by Phillips et al (41). They performed an objective prospective study in cirrhotics with PCS and a randomly selected control group of similar unshunted cirrhotic patients. All 108 patients in the study met the following criteria: 1) histologic diagnosis of esophageal varices; 2) no prior hemorrhage from varices; 3) presence of overt ascites; 4) portal venous pressure more than 200 mm Hg; 5) reasonable risk for elective surgery; and 6) less than 65 years old.

Forty-eight patients underwent elective PCS and 60 remained unoperated. The diagnosis of peptic ulcer disease was made by unequivocal demonstration of an ulcer on x-ray, endoscopy, surgery, or autopsy. Acute stress ulcers were not included.

The patients were followed for 44 months. Seven of the 60 controls developed peptic ulcers (11.7%); 6 of the 48 shunted patients developed peptic ulcers (1.5%). There was no significant difference between the two groups. The majority of the shunted patients developed duodenal ulcers while almost half of the controls developed gastric ulcers, but this was also not statistically significant. There was also no statistically significant difference between the two groups in terms of the number of patients with ulcers who developed complications from them. They attribute the lack of increased ulcer formation to the fact that a patient with portal hypertension develops an extensive series of collaterals between his portal and systemic circulations, and a PCS may only redirect the blood flow between them while not markedly increasing it.

From this study one can conclude that PCS does not lead to an increased incidence of peptic ulcer disease. However, through the investigation of this problem, physiologists were able to develop a more extensive understanding of the control of gastric secretion.

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Student Section

Autoimmune Factors in Inflammatory Bowel Disease

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Introduction

Despite decades of intensive laboratory investigations punctuated by frequent enthusiastic reports, the origins of inflammatory bowel disease (IBD) remain enigmatic. Theories, concerning the etiology of both chronic ulcerative colitis (CUC) and Crohn's disease (CD) have at various times emphasized allergic, psychosomatic, vascular, genetic, environmental, enzymatic, microbial, and immune factors. Perhaps the most intriguing of these approaches is the speculation that IBD represents an autoimmune disease process. According to this theory, the pathophysiology of IBD represents the effects of an *endogenous immune response to an endogenous antigen.* While there is no single piece of evidence providing conclusive proof that IBD represents an autoimmune process, there are a number of clinical and experimental observations which are strongly suggestive of such a mechanism. These observations, coupled with the unique apparatus of the immune system in the gastrointestinal tract and recent theoretical advances in immunology, provide the basis for a cogent, though still speculative, hypothesis that IBD represents an immune response directed against autologous gastrointestinal antigens.

General Features of Autoimmune Disease

Consideration of the hypothesis that IBD represents an autoimmune disease process requires a well-defined conceptual framework delimiting the properties of autoimmune disease. Sell proposes the following six characteristics as being ideally identifiable in all immune processes and which should be sought for in all diseases of suspected autoimmune etiology (1):

1. A well-defined immunizing event (infection, vaccination, etc.)
2. A latent period (usually 6–14 days)
3. A secondary response (a more rapid and intense reaction on second exposure to the antigen)
4. An ability to transfer the sensitive state with cells or serum from an infected individual to a normal individual

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5. A specific depression of the sensitive state by large amounts of antigen (desensitization)
6. Identification and isolation of the antigen in pure form and chemical characterization of the antigen

As Sell is quick to point out, all, or even a few, of these criteria can rarely, if ever, be established for human diseases, although these criteria may be met in certain experimentally induced autoimmune diseases. One may suspect an autoimmune process in diseases which fail to meet these criteria. Presumptive findings consistent with, but *not* strong evidence for, an autoimmune mechanism in a human disease state include (2):

1. A morphologic picture consistent with known allergic reactions
2. The demonstration of antibody or a positive delayed skin reaction.
3. A depression of complement during any stage of the disease
4. A beneficial effect from agents known to inhibit some portions of an allergic reaction (steroids, radiation, anti-metabolites, etc.)
5. An association with other possible autoimmune diseases
6. Identification of a reasonable experimental model in animals that mimics the human disease
7. An increased familial susceptibility to the same or other autoimmune diseases
8. An association between the disease state and specific HL-A (human histocompatibility antigen) types

The reason that these criteria provide only weak evidence is that they fail to distinguish between immune phenomena as causal agents of a disease process and immune phenomena as epiphenomena of an ongoing disease process. Most of the observations concerning IBD suffer from this weakness. For example, the demonstration of anti-colon antibodies in CUC does not establish these antibodies as being the causal agent of mucosal ulceration. Rather, these antibodies might appear secondarily in response to existent, nonimmunologic damage to colonic epithelium. On the other hand, an immune process developing secondarily might contribute to the nature of the tissue reaction to its chronicity.

Possible Mechanisms of Autoimmunization

Perhaps the greatest stumbling block to the concept of autoimmune disease is the difficulty in accounting for a mechanism by which the body becomes immunized to autologous tissue components. If such autoimmunization is possible, why is it restricted to a relatively small human subpopulation? Several mechanisms have been proposed:

1. *Sequestered antigen:* An autologous antigen might never encounter antigen receptive lymphoid cells until after the period of embryological development in which tolerance to self normally develops. The tissues involved in autoimmune disease are usually derived from ectoderm or endoderm and are regarded as foreign by the immune apparatus which is

mesodermal in origin. These autoantigens are absent during the immune neutral period of development and therefore fail to induce tolerance like other body antigens. Blood-tissue barriers normally prevent these substances from ever reaching the circulation and the immune apparatus. When viral infection, injury, or other episodes cause breakdown of blood-tissue barriers, with release into circulation, allergic reactions may occur (3).

2. *Altered antigen:* Some chemical, physical or microbiologic event might alter an antigen so that it is no longer recognized as self by the immune system. The consequence would be an immunologic reaction against the altered antigens. By immunizing animals with aqueous homologous thyroglobulin to which arsanilic or sulfanilic acid has been coupled, it is possible to produce experimental allergic thyroiditis (4). Even more intriguing is the possibility that viral infection might lead to surface antigen modification by means of peplomers inserted into the host cell membrane. A dramatic example of the expression of a new surface antigen on viral infected T-cells has been described by Obota et al: (5).
3. *Release of forbidden clones:* Largely based upon the clonal selection theory of Burnet (6), this theory states that an alteration occurs, not in the tissue in which the lesion appears, but in the cells of the immune system. This alteration results in a failure to recognize normal antigens as self with proliferation of a clone of lymphocytes directed against self. There is little experimental evidence to support this theory other than the occasional association between putative diseases of autoimmunity and thymoma.
4. *Symbody deficiency:* Certain tissues including the trophoblast-bearing paternal antigens and colonic mucosa bearing deposited bacterial antigens are viewed by the immune system as representing non-self. However, tolerance develops to these antigens by virtue of the immunosuppressive action of certain immunoregulatory alpha globulins (IRA or symbody) and secretory immunoglobulin. A masking complex consisting of the foreign antigen, a lipid receptor, a plasma glycoprotein, and secretory IgA prevent the lymphoid system from recognizing the foreign antigen (7). Any failure of the symbody system to adequately cover these partially foreign host antigens leads to a breakage of tolerance and autoimmune disease. Such a failure might result from a deficiency of specific secretory IgA molecules or deficiency of IRA. At the present time this theory remains highly speculative.

Immunologic Apparatus of the Gastrointestinal Tract

The gastrointestinal tract is particularly well equipped to generate immunologic reactions both locally and systemically. Local collections of lymphoid tissue underlie the submucosa along the entire length of the GI tract. In some areas, the collections become large enough to be indentifiable as distinct lymphoid organs, for example, tonsils, appendix, and Peyer's patches. These tissues consist almost entirely of lymphoid follicles, with little or no medullary element. In this respect they differ anatomically from the peripheral lymphoid organs—lymph nodes and spleen. Gastrointestinal lymphoid tissue is believed necessary for development of the antibody forming organs (germinal centers, plasma cells). According to current theory of lymphocyte ontogeny, potentially immunocompetent cells must come into contact with, or be affected by, products of endodermal tissue (8). The embryonal gut-associated (endodermal) lymphoid tissue, commonly referred to as central lymphoid tissue, consists of the thymus, tonsils, appendix, Peyer's patches, and, in the fowl, the bursa of Fabricius, a hindgut organ. The remaining lymphoid tissue, including lymph nodes and spleen, constitutes peripheral lymphoid tissue (9).

Lymphoid organs may be further divided on the basis of function. The thymus is believed to be necessary for the proper development of cellular (delayed type) immune mechanisms mediated by lymphocytes. Other endodermal areas have been implicated as being necessary for the development of humoral (antibody-mediated) immune capacity. It has been shown that removal of the bursa of Fabricius in chickens leads to complete deficiency of humoral immunity while cellular mechanisms remain intact (10). Although no clear bursa equivalent has been demonstrated in man, it has been hypothesized that bursal function is subserved by GALT (gut-associated lymphoid tissue) consisting of tonsils, appendix, Peyer's patches, and possibly mesenteric lymph nodes and lamina proprial lymphocytes (11).

It is thought that GALT plays a primary role in establishing immunity to infectious agents entering the body through the oral cavity. Observations on the intestinal mucosa of germ-free animals contrast sharply with those bearing normal intestinal flora in that there is a marked diminution in the number of immunocytes at all levels of the intestinal tract (12). The scarcity of lymphocytes in the germ-free state has led to the use of the term "physiologic inflammation" for the rapid accumulation of mucosal lymphocytes upon exposure to exogenous microbial antigens (13).

Lymphocytes in Intestinal Lamina Propria

The mechanisms by which lymphocytes extravasate to specific peripheral and gut-associated tissue compartments are poorly understood yet are fundamental to the comprehension of general immunocompetence and of specific clinical problems such as allograft rejection and IBD. Gowans and Knight initially observed that intravenously injected rat thoracic duct lymphoblasts migrated in large numbers to the mucosa of the small intestines of syngeneic recipients; they postulated that these lymphocytes localized to the site of enteric antigens to which they had already responded (14). Later work showed that lymphoblasts obtained from mice stimulated with nonenteric antigens (14–16) or from unsuckled, presumably germ-free, neonatal mice (17), also showed avidity for the small intestine, indicating that the ability of an immunoblast to "home" to the gut is independent of its immunologic specificity.

Recent work suggests that lymphocytes localized in the intestinal lamina propria constitute a unique lymphoid subpopulation. Analysis of the tissue localization of injected radioactively labeled lmphocytes has been a useful technique for distinguishing lymphoid subpopulations of the basis of differential patterns of migration (18). Utilizing this technique, it has been shown that there is a distinct subpopulation of lymphocytes, definable by their property of labeling with IUDR (a thymidine analogue specific for cells duplicating their DNA) which possess migrational specificity for the lamina propria of stomach, jejeunum, ileum, and large bowel. This gut-seeking population can be found within peripheral nodes, mesenteric nodes, Peyer's patches, spleen, and thymus and consists of both T and B cells (19).

Conversely, there is evidence to suggest that enteric immunocytes communicate both with central and peripheral lymphoid organs. Since splenic plaque-forming cells (B cells) can be indentified following oral immunization with most antigens (20, 21), it is likely that either free antigen or cells originating in the GI mucosa (or both antigen and cells) reach the systemic circulation. Response to oral antigens might involve several populations of cells (22):

1. cells located in the GI tract
2. cells encountering antigen in the GI tract but which have left the GI tract and migrated to other lymphoid tissues
3. cells stimulated by absorbed antigens in peripheral sites such as the spleen

Evidence for the involvement of each of the three populations has been reported in several different species with several antigens. Furthermore, lamina propria cells are able to respond both to intravenous and intrajejeunal injection of sheep red blood cells (SRBC) as evidenced by the formation of antibody-producing cells (23). Thus, there is an intimate link between systemic immune responses and immune responses occurring in the intestinal mucosa.

The function of gut-seeking lymphocytes remains to be analyzed. Immunofluorescent staining of the lamina propria along the entire length of the GI tract has shown that the majority of the immunoglobulin-producing cells in this site synthesize IgA (24, 25). The significance of gastrointestinal IgA will be discussed under a later heading.

The nature of the T cell population homing to the gut remains problematic. There is data to suggest that immature thymocytes possess migrational specificity and that these cells may leave the adult thymus and colonize gastrointestinal tissue (26). An intriguing possibility is that these T cells serve as "helper" cells for IgA synthesis. There are several lines of evidence suggesting a distinct relationship between IgA and T cells. Thymectomized rats show a selective deficiency in their ability to form antibodies of the IgA class against bovine serum albumin (BSA) (27), as well as a relative deficiency in total IgA (28) when compared to non-thymectomized controls. There was no deficiency in the IgG or IgM class. Similar observations were made measuring the response to BSA-arsanilic acid by neonatally thymectomized rabbits. Thus, at least for certain antigens, the IgA antibody response is more dependent upon T cell function than the antibody response in other immunoglobulin classes.

There are several lines of clinical evidence which support the experimental observations. The majority of the patients with ataxia telangiectasia have a selective deficiency of IgA along with absolute T cell deficit. Schiff and coworkers, in a study of IgA deficient patients have found a depression in the number of T cells as measured by the SRBC rosette technique (29). In addition, there have been reports of a variable response to phytohemagglutinin in patients with selective IgA deficiency (30). The possibility exists that in the GI tract there are helper cells for the production of each class of immunoglobulin and that selective IgA deficiency represents a dyscrasia of T cells rather than B cells. It is interesting to note that the majority of patients with IgA deficiency have normal numbers of circulating B cells (31), and these cells are able to synthesize IgA if stimulated by pokeweed mitogen (PWM) (32).

Peyer's Patches

The functions of Peyer's patches are not yet well known. For some time there was much support for the theory that Peyer's patches represent the human equivalent of the avian bursa of Fabricius. It is now known that this is not the case because Peyer's patches contain large numbers of T cells that have migrated from the thymus (33). Moreover, although present at birth, Peyer's patches are poorly formed and develop T and B populations after birth in a manner similar to that of peripheral lymph nodes. Peyer's patches are unique, however, because although they contain both T and B cells, they are unable to mediate graft-vs-host or allogeneic reactions (typical T cell functions) or to manifest a B cell response when immunized in vivo. These differences may result either from a lack of antigen trapping mechanisms and the absence of other cell types (i.e., macrophages) or the factors required for the induction of T and B cell function (34). An alternative possibility, which cannot be excluded, is the presence of a high density of "suppressor" cells within Peyer's patches.

Craig and Cebra have shown that Peyer's patches represent an enriched source of IgA precursor cells and speculate that the function of this organ may be to "seed" the gut with cells which proliferate within the lamina propria and mature into IgA-producing plasma cells (35). Lymphocyte migration studies suggest that there are two distinct lymphocyte populations which home to Peyer's patches—one similar to the population recirculating through lymph nodes by means of postcapillary venules and one with a specificity for the lamina propria (36). As such, Peyer's patches may represent a linkage between gastrointestinal and systemic immune systems.

The Secretory Immune System of the Gastrointestinal Tract

While IgG is the dominant circulating antibody in serum and in other fluids that are completely contained within the body, the secretory gamma A antibodies are quantitatively and probably qualitatively dominant in fluids bathing the systems which are in continuity with the external environment. Saliva, tears, breast milk, colostrum, gastrointestinal secretions and the mucinous secretions of the respiratory and genitourinary systems are all rich in IgA. Thus IgA is uniquely situated to provide the first line of immunologic response to external pathogens (37). What remains problematic is the mechanism by which IgA is effective in regulating the gastrointestinal flora. Secretory IgA antibodies do not fix complement and it is generally believed that lysis of a bacterium by antibody requires the action of complement. Nevertheless, there are several important mechanisms by which IgA can effectively regulate microbial populations.

1. There is substantial evidence indicating that secretory antibodies can neutralize viruses at their portal of entry (38). Resistance to poliomyelitis virus following oral immunization correlates better with titers of antibodies in secretions than with those in serum (39).
2. IgA may be able to activate complement via the alternate pathway or C-3 shunt. Evidence for this mechanism is restricted to in vitro work in which complement components were supplied in concentrations that were significantly higher than those occurring in vivo (40). Further work is needed to establish if this mechanism actually occurs in the human gastrointestinal tract.
3. Recent reports suggest that antibodies of the IgA class are capable of inhibiting the adherence of bacteria to epithelial cells (41). For bacteria to colonize a mucosal organ effectively, the organism must first adhere to the mucosal epithelial cell. If the initial attachment is prevented by antibody, the organism would be mechanically removed by secretions before colonies sufficiently large to produce a detrimental effect could develop.
4. Secretory IgA might prevent the mucosal absorption of dietary or microbial antigens. Suspicion for this role came from the observation the patients with selective deficiency of IgA have high titers of serum antibodies against milk proteins (42). Particularly prominent are IgG antibodies against bovine milk IgM. Such antibodies are rarely present in controls. More concrete evidence was obtained by Walker (43) who showed that in rats the extent of absorption of proteins such as BSA and HRP can be influenced by pre-immunization with these antigens. The effects of immunization were antigen specific, i.e., immunization with BSA affected absorption of BSA but did not affect absorption of HRP. An association has been noted between selective IgA deficiency and autoimmune disease (44). It is possible that patients with defective secretory immune systems absorb large amounts of exogenous antigens (i.e., E. coli antigens) that cross react with certain endogenous antigens leading to autoimmune disease.
5. Secretory IgA may serve a synergistic role with immunoregulatory alpha globulins (symbodies) in preventing epithelial surfaces bearing bacterial antigens from being recognized as foreign (See p. 604).

Clinical Features Suggesting Autoimmune Mechanisms

One of the strongest arguments in favor of the involvement of immune mechanisms in IBD comes from a consideration of the clinical presentation and course of the disease process. Several observations have been made which when considered jointly are strongly suggestive of an autoimmune process:

1. IBD runs a chronic relapsing course not unlike that of rheumatoid arthritis and the other connective tissue diseases for which there is good evidence for an immunopathologic basis.
2. In CD, resection of the diseased tissue may produce a remission period lasting ten years with subsequent recurrence of pathology. Such a course suggests retention of the disease process, either in terms of a dormant pathogen or in the form of immunologic memory.
3. A frequent personal and family history of allergic disorders (45).
4. Occasional occurrence of IBD in association with other disorders linked with immunologic mechanisms (i.e., eczema, rheumatic fever, myocarditis, autoimmune hemolytic anemia, thrombocytopenic purpura) (46).
5. The multiplicity of connective tissue type diseases in the occasional patient with IBD. A case has been reported of a young woman with ulcerative colitis, arthritis, scleroderma, pneumonitis, and febrile episodes resembling SLE (47).
6. The beneficial response to corticosteroids and immuno-suppressive drugs such azothioprine and azulfadine. These drugs are capable of providing considerable palliation and occasionally complete remission from an acute flare up of disease, although the long term effects are uncertain and these drugs are generally not thought to alter the ultimate course of the disease process. Nevertheless, the initial response to immunosuppressive therapy strongly suggests modification of an underlying immunopathologic process (48).
7. Reports of familial occurrence of both CUC and CD are consistent with vulnerability to disease mediated by immunogenetic factors.
8. Extraintestinal manifestations of IBD affect almost every system in the body. Although a common cause remains to be established for these manifestations, immunological mechanisms seem to be the best hypothesis to link them with the intestinal disease. Common manifestations include (49):
    - Liver: pericholangitis, active chronic hepatitis, primary biliary cirrhosis
    - Skin and mucous membranes: erythema nodosum, pyoderma gangrenosum, erythema multiforme, apthous stomatitis, Behçet's syndrome
    - Blood: Coomb's positive hemolytic anemia, hypergamma-globulinemia
    - Occular system: iritis, iridocyclitis, episcleritis, conjunctivitis
    - Bones and joints: arthritis, anklyosing spondylitis, tenosynovitis, sacroileitis
    - Other: pericarditis, pulmonary vasculitis, thrombophlebitis, pancreatitis, glomerulonephritis.

Histologic Features Suggesting Immune Mechanisms

The most characteristic histologic features of CD are "the transmural inflammation (in the form of chronic inflammatory infiltrates and fibrosis) affecting all the layers to the serosa and the development of non-caseating granulomas closely resembling those of sarcoidosis . . . the resemblance to sarcoidosis is complete with the production of multinucleated giant cells, some of which contain Schauman bodies" (50). In addition to the presence of granulomas there is a marked lymphoid hyperplasia with follicles seen throughout all layers of the bowel wall as well as in the Peyer's patches and other gut-associated lymphoid tissue. In a few patients the lymphoid hyperplasia is so great that the pathology simulates a malignant lymphoma (51). Eosinophils may be seen in the submucosa in addition to more numerous lymphocytes and plasma cells. Scant macrophages are present containing PAS-positive hyaline material, possible emanating from an Ag-Ab reaction. Although many of these are nonspecific they are suggestive of mechanisms involving cellular immunity, delayed hypersensitivity, and allergy.

The lesions of ulcerative colitis are less distinct consisting entirely of nonspecific inflammation and scarring of the mucosal and submuosal layers. There is some evidence suggesting that the initial ulceration or micro-abscess at the depth of the crypts in the mucosal gland is preceded by an acute vasculitis. Thus there is a suggestion that immune-mediated vascular lesions precede and lead to infarction necrosis of the bowel mucosa (52). As with CD, the presence of lymphocytes, plasma cells, eosinophils, and mast cells in the lesions of CUC has been used as evidence for the involvement of immune mechanisms.

Humoral Features in IBD

Anti-colon antibodies: Circulating hemagglutinating antibodies to colonic mucosa were first reported in patients with CUC in 1958 by Cornelius (53). This observation has since been confirmed utilizing a variety of techniques including precipitation, hemagglutination, and immunofluorescence to demonstrate antibodies in the sera of some CUC patients which react with antigens obtained from different colonic tissues and tissue extracts, germ-free rat feces, or both (54, 55). Although early studies (56, 57) failed to detect circulating anti-human colon antibodies in the sera of patients with CD, more recent work (58, 59) has demonstrated hemagglutinating antibodies in sera from patients with either regional enteritis or CD of the colon. The serum antibodies were found with equal frequency in patients with CUC and CD regardless of the site of disease involvement.

Immunochemical techniques have been utilized to determine the chemical nature of the "colon antigen." The following characteristics have been established (60):

1. consists of immunologically heterogenous carbohydrates, predominantly mucopolysaccharide, possibly involving gastrointestinal mucopolysaccharides as found in mucin.

2. the main sugars are galactose, glucose, fucose, mannose and a lipopolysaccharide, possibly identical with certain hexoses found in enteric organisms
3. heat stable at pH 7–9, water soluble, slowly destroyed by mild acid hydrolysis, and more rapidly destroyed by oxidation by periodate
4. probably localized in cytoplasm-sera from patients with CUC binds to the cytoplasm of epithelial cells of colonic mucosa
5. gastrointestinal-specific but not colon-specific—similar to gastrointestinal-specific acid mucopolysaccharides limited to mammalian species in association with secretory regions of mucosal cells throughout the GI tract

Because colon antibodies are mucopolysaccharides which are widely distributed, the suggestion has been made that the same antigenic determinants are present in other tissues and represent a tissue group isosystem (61). It has been postulated that the anticolon antibodies in the sera of patients with IBD as well as in the sera from healthy subjects and patients with non-gastrointestinal disease may represent naturally occurring isoantibodies akin to the ABO system of human erythrocytes (62). Support for this theory comes from the observation that there is an increased incidence of anticolon antibodies in the sera of apparently healthy relatives of patients with CUC (63, 64).

Studies of human sera giving positive immunofluorescent tests for anti-rat colon antibodies have demonstrated that the antibody population is extremely heterogenous. Activity has been associated with IgG, IgM, and IgA with almost equal frequency (65). In some instances circulating anti-colon antibodies were associated with secretory IgA, but no activity has been found for IgD or IgE.

At the present time it is not known whether anticolon anti-bodies are of any significance in the pathogenesis of IBD. These antibodies have been shown capable of significantly influencing the rate of DNA synthesis, as measured by thymidine uptake, in colonic epithelial cells in guinea pigs and rabbits (66). However (67–70):

1. Anti-colon antibodies are not cytotoxic to human fetal colonic cells in tissue culture
2. Specific antibodies have not been demonstrated in colonic tissues in tissue culture
3. It has not been possible to correlate the titer of anti-colon antibodies with the severity, duration, course, or extent of the disease or with the presence or absence of extracolonic manifestations
4. Antibody titers are not affected by treatment with steroids. Thus it seems likely that these antibodies are not a major factor in the pathogenesis of IBD. Anti-colon antibodies are significant however, in that they provide a means by which immunoreactive colonic antigens may be better localized and defined.

Antibacterial antibodies: The observation has been made that shared antigenic determinants exist between antigens in germ-free rat colon and feces and a lipopolysaccharide found in E. coli 014 (71). This lipopolysaccharide contains, in addition to endotoxin, large amounts of a heterogenetic antigen (72) which is a constituent of most Enterobacteriaceae strains. Antibodies reactive with gut mucosa can be produced in animals by injecting nonviable bacteria in Freund's adjuvant (73) or E. coli isolated from the feces of patients with CUC (74). Furthermore, the detection of anti-rat colon antibodies in the sera of patients with CUC may be inhibited by preabsorption with extracts of E. coli 014 as well as with antigens obtained from germ-free rat, rabbit, and human colon (75, 76).

It has been suggested that stimulation by cross-reacting bacterial antigens may serve as a mechanism by which the body becomes immunized to endogenous colonic antigens (77–79). Weinstein (80) has proposed that E. coli 014 may be responsible for CUC in the same manner that group A beta-hemolytic streptococci act in rheumatic fever. Although attractive, there are several problems with this hypothesis. Sera from CUC patients and controls are indistinguishable with respect to antibody titers to several common strains of E. coli (81), while it has been observed that there are comparable hemagglutinating anti-E. coli 014 antibody titers in the sera of patients with CUC and controls (82, 83). A recent study (84) has verified these observations utilizing an extremely sensitive radioimmunoassay for antibodies against a nonpathogenic strain of E. coli. Although there was no quantitative difference in antibody titers discernible between CUC patients and controls, there was a qualitative difference in the antibody response. Patients with CUC tended to have lower concentrations of anti-E. coli antibodies of the IgG and IgA classes as compared to controls, and in three patients there was no detectable IgG anti-E. coli antibody in the serum. The significance of this observation has not yet been established nor has a similar analysis been applied to titers of secretory immunoglobulins.

Since the heterogenetic antigen is present in healthy individuals, additional factors are necessary to explain the development of autoimmunity in IBD. Data showing elevated titers of antibody against both germ-free colon and E. coli 014 in sera from female relatives of patients with CUC are compatible with the existence of predisposing genetic factors (85). Escherichia coli 014 and germ-free rat colon share antigens with both human kidney and group A nephritogenic cocci (86) as well as with A and B blood group-active polysaccharide antigens (87). The possibility that the colonic antigen is part of a multitissue isosystem has been discussed (see page 611).

Another mechanism by which bacteria might elicit an autoimmune response is by damaging mucosal cells and altering their antigenic configuration or by attaching in hapten-like combination and contributing to colonic antigenicity (88). Gram-negative bacteria are rich sources of endotoxins, known to be effective antigens and adjuvants. A local Shwartzman reaction related to bacterial endotoxin may be an additional immunologic reaction contributing to the tissue reaction in IBD (89).

Miscellaneous antibodies: Other circulating reactants which have been occasionally detected in sera from patients with IBD include antinuclear factors, rheumatoid factor, anti-erythrocyte antibodies, antibodies to gastric and small intestinal mucosa, gastric parietal cell antibodies, antibodies to thyroglobulin, adrenal antibodies, precipitins to pancreatic homogenates, antibodies to phenol-water extracts of liver or kidney, and antibodies to reticulin (90). The etiology of these antibodies and their contributions to the intestinal and extraintestinal manifestations of IBD are not well understood.

Serum and tissue immunoglobulin levels: Analysis of the levels of serum immunoglobulins in patients with IBD has not yielded dramatic results. There has been little evidence demonstrating statistically significant differences between the mean level of the major immunoglobulin classes in patients with IBD when compared to controls. Although some researchers have found an elevation of serum IgA concentrations to be a frequent feature of CUC (91), the coexistence of CUC with selective IgA deficiency has also been reported (91). Elevated serum secretory IgA levels have been reported in several patients with CUC (93, 94). This observation cannot be explained nor can the observation that even higher levels have been observed in patients who have undergone colectomy (95). It is possible that in fulminant disease IgA-producing cells migrate to intestinal tissue via the circulatory system and that increased serum levels of secretory IgA occur secondary to the loss of homing sites.

Quantitative analysis of tissue immunoglobulin levels in the rectal mucosa has provided equally confusing results. In one study (96), the population density of IgA cells in the lamina propria of rectal mucosa in patitents with CUC was found to be lower than in persons with normal rectal tissue. In another study (97), rectal mucosal biopsies from untreated patients with CUC were found to have a significant increase in IgA and IgA synthesis in comparison with normal controls and patients with bacterial enterocolitis. In view of the unique role played by the secretory immunoglobulin system in the gastrointestinal tract, more attention needs to be directed toward direct measurements of tissue immunoglobulin levels. It is possible that better correlations will be found between disease activity and tissue levels of immunoglobulin than has been found to date for serum levels of immunoglobulins.

Cellular Factors in IBD

Perhaps the most important experimental observation concerning IBD is the recognition that peripheral lymphocytes from patients with IBD are cytotoxic in vitro for autologous or allogeneic colon epithelial cells (98, 99). The phenomena of lymphocyte-mediated target cell destruction has been recognized in a number of clinical situations, most notably allograft rejection and tumor immunity, and experimental autoimmune disease models. As a result of this experience, a number of mechanisms have been demonstrated by which lymphocytes can exert a cytotoxic effect. These mechanisms may be summarized as follows (100):

1. Lymphocytes from sensitized donors may be cytotoxic for target cells posessing membrane-associated antigens.

2. Lymphocytes sensitized to soluble antigens and reexposed to such antigens, or activated by nonspecific mitogens, may exert a cytotoxic effect on target cells antigenically unrelated to the sensitizing antigen or mitogen due to the release of a nonspecific lymphotoxin.
3. Unsensitized lymphocytes from normal individuals may be induced to cause cytolysis of target cells in the presence of specific anti-target cell antibodies.
4. Unsensitizied lymphoid cells may lyse target cells coated with complement components.

Although it is possible that each of these mechanisms is operational in IBD, most of the evidence points to the predominance of lymphotoxin-mediated target cell destruction. Largely as a result of the work of Shorter and colleagues, the following characteristics have been demonstrated for lymphocyte-induced toxicity for colon epithelial cells in patients with IBD (101a):

1. present in virtually all patients with IBD including patients both with CUC and CD
2. present only in IBD—lymphocytes from patients with a variety of inflammatory and neoplastic lesions of the small and large bowel as well as from patients with several connective tissue disorders are not cytotoxic
3. lymphotoxin release is specific for enterobacterial antigens or colon epithelial cell antigens
4. cytotoxic lymphocytes disappear within ten days after removal of all diseased tissue
5. cytotoxic lymphocytes persist during clinical remissions
6. cytotoxicity may be conferred upon normal lymphocytes by preincubation with E. coli 0119:B14 phenol-ether extract or the serum IgM fraction from patients with IBD
7. cytoxicity of IBD lymphocytes may be blocked by:
    -preincubation of lymphocytes with coliform extract
    -preincubation of lymphocytes with anti-IgM
    -preincubation of lymphocytes with isologous IBD serum
    -addition of anti-lymphocyte serum to the system
    -preincubation of colon cells with anti-colon antibodies
8. the cytotoxin is present in the cells preformed since cell free extracts of disrupted lymphocytes from patients with IBD are equally cytotoxic
9. the addition of complement is not required for the reaction to occur

Stobo and Shorter have recently presented evidence (101b) suggesting that the cytotoxic cells found in the peripheral blood of patients with IBD may not belong to either of the classical T or B lymphocyte subpopulations. Cytotoxic lymphocytes do, however, bear surface receptors that combine with the Fc portion of immunoglobulin. The existence of this Ig receptor provides a plausible basis for the role of IgM (see 6 and 7 above) in the cytotoxic effect. It has been suggested that patients with IBD have circulating lymphocytes bearing cytophilic IgM which react with E. coli 0119:B14 antigen or allogeneic colon epithelial cells resulting in the release of nonspecific cytotoxin causing target cell destruction (102). The role played by IgM has not been well established, nor is it known whether the lymphocyte-associated IgM is produced by the lymphocytes themselves or if it is produced elsewhere and passively absorbed.

Analysis of the circulating lymphocyte populations in patients with IBD has shown a decrease in the relative proportion of T cells and an increase in the relative proportion of B cells (103). One must exercise caution in interpreting these findings. Assays used to quantitate T cells, either SRBC rosette formation or depletion by anti-O serum, are specific for mature recirulating T cells. It has been demonstrated in mice that there is a subpopulation of thymocytes, definable by their property of labeling with IUDR, which are capable of specific homing to intestinal lamina propria (104). These cells are not mature T cells, but rather represent immature precursors which do not necessarily bear the full complement of T cell antigens. Thus it is possible that some or all of these cells will not be measured by conventional T cell assays. The decrease in T cell proportion may therefore reflect a shift in the T cell pool from one consisting largely of mature cells to one with a significant number of immature precursors. Conversely, the observed increase in B cells may reflect surface binding of IgM by these immature thymocytes, and inclusion in a B cell population measured by binding of polyvalent anti-Ig. Further analysis of the circulating lymphocyte populations in IBD is required as well as analysis of the kinetics of lymphocyte production and release by the thymus in control and disease states.

Anergy and IBD

One of the most confusing experimental observations in IBD has been that of a possible impairment of cell-mediated immune responses in patients with IBD. Divergent results have been obtained studying dermal reactivity to PPD-S, mumps antigen, fungi, and DNCB as well as lymphocyte responsiveness to mitogens such as PHA and CON A. Shorter and Watson have reviewed the literature concerning anergy in IBD and have concluded that the available evidence "strongly supports the existence of some type and degree of anergy as measured by lymphocyte hyporesponsiveness to nonspecific mitogens in patients with CD and probably CUC as well " (105). However these authors are quick to point out that the meaning of such anergy is unclear, and in light of the fact that anergy occurs in a wide range of clinical disorders and is unrelated to clinical events, it is likely that lymphocyte hyporesponsiveness to mitogens is an acquired epiphenomena and is of no primary or pathogenic importance.

Transmissable Agent in CD?

A microbial etiology for IBD has long been an attractice hypothesis. The disease process involves mucosal surfaces harboring a complex microbial population and constantly exposed to a contaminated fecal stream. Furthermore, histologic changes which characterize both CUC and CD closely simulate lesions of infectious origin. CUC closely mimics bacillary dysentery.

Both shigella and multiple species of enteric gram positive cocci have been shown to cause colonic ulceration and bloody mucoid diarrhea in rabbits exposed to intravenous challenge. The hallmark of CD is a sarcoid-like noncaseating epithelioid granuloma in which a multinucleated Langerhans-type giant cell is surrounded by and aggregation of large epithelioid mononuclear histiocytes. These histologic changes combined with the clinical picture of ileal inflammation, fistulization, and stenosis closely mimics the manifestations of intestinal tuberculosis. Exhaustive microbiologic and serologic studies have been performed on patients with CD in an unsuccessful effort to identify acid-fast bacilli, spirochetes, parasites, or granuloma producing viruses. The failure of these efforts do not necessarily rule out a microbial agent in CD, but rather may reflect a situation similar to syphilis or leprosy in which the established pathogen cannot be recovered in artificial media (106).

It is in this context that the findings of Mitchell and Rees (107) have generated considerable interest. These authors found that mice inoculated in the rear hindpads with homogenates from diseased lymph nodes of patients with CD developed epithelioid granulomas at the injection site. This work was successfully reproduced by Cave and Mitchell (108) who reported that lesions similar to those of CD could be produced by injecting lymph node homogenates directly into the ileal wall of rabbits. Sachar and Taub (109) have further demonstrated that granuloma-eliciting material can be found not only in affected lymph nodes, but also in affected tissues of patients either with CUC or CD. This property was not restricted to portions of bowel containing granulomas because no granulomas were present in the tissues resected from CUC patients. Furthermore, in Crohn's patients, where histologically "normal" tissue was obtained from the margins of the surgical resection, granuloma-eliciting activity was still present.

One must exhibit extreme caution in interpreting the results of these experiments. Transmission of granulomas is not necessarily equivalent to transmission of IBD. Furthermore, although these results are compatible with a microbial agent present in the tissue homogenates, the transmissible agent need not be an intact virus or bacterium capable of reproduction. The agent might be an antigen, representing either bacterial or viral fragments or a normal tissue component which has been altered by hapten-like attachment of viral or bacterial products. Alternatively, the injected homogenate may contain nonspecific chemical mediators of inflammation such as lymphocytoxin and MIF (110). The granuloma thus produced in the laboratory animal need not bear any relation to the antecedent human disease producing these chemical substances. Finally, the tissue inoculum undoubtably contains lymphoid cells or at least lymphoid RNA, and the reaction being transmitted might be an immunologic rather than infectious one (111). Papatestas and Kark (112) point out that in the original Mitchell and Rees experiment 18 out of 28 normal mice developed granulomas when injected with CD homogenates while in immunologically deficient mice (thymectomy + whole body irradiation) injected with the same tissue homogenates, only 9 of 28 developed granulomas. The immunodeficient animals were thus less susceptible to the transmissible agent than normal animals. This observation is more in accordance with a cell-mediated immune mechanism than an infectious mechanism where one would expect an opposite effect. On the basis of this observation these authors are currently exploring the possible role of thymectomy in the treatment of CD (113).

The Shorter Hypothesis

Shorter and associates have recently proposed a working model for the etiopathogenesis of IBD which takes into account many of the diverse observations which have been presented. This hypothesis rests upon two basic assumptions: 1) that CUC and CD represent parts of the spectrum of a single pathogenetic process, and 2) IBD results from the establishment of hypersensitivity to bacteria antigens normally present in the gastrointestinal tract of the affected individual (114).

The first assumption is based on the following observations 1) the often merging histopathologic characteristics of the two disorders, 2) their similar distributions of age, sex, and race, 3) the extraintestinal manifestations shared by each, 4) the familial incidence for both is interdependent, and 5) their common immunologic features (115).

The second assumption is based upon the "near-universal" existence of immunity to a coliform antigen as suggested by the finding that lymphocytes from most normal individuals produce macrophage inhibitory factor on exposure to a phenol-water extract of E. coli 0119:B14. Thus most people are properly primed to mount an immunologic response to enterobacterial antigens given the proper antigenic challenge. Such a challenge might occur secondary to any process which impairs the normal mucosal block to the uptake of bacterial antigens. Such processes might include gastroenteritis, bacillary dysentary, amebic colitis, ischemic episondes, dietary toxins, or even metabolic alterations induced by psychologic stress (116).

Once sensitization and rechallenge occur the interaction of enterobacterial antigens and/or related colonic antigens with sensitized lymphocytes causes the latter to release biologic mediators such as lymphotoxin. Lymphotoxin then acts nonspecifically, affecting any cell within its range of activity. If release of lymphotoxin occurs at the mucosal level a relatively superficial type of colitis might be expected to occur, while release within the submucosa might cause a transmural or granulomatous inflammation. Within this hypothesis, when the lymphocytes interact primarily with the membrane-associated antigens of colonic epithelial cell, the feature of CUC would predominate. If however, cross-reacting bacteria gained access to the submucosa, the transmural features of CD would emerge. Thus, the variable and often merging pathologic features noted in the colon in these two diseases are explained (117). Clinical observations supporting this hypothesis include (118):

1. The location of the lesions in more than 90% of cases of CD and in all cases of CUC in those portions of the gut exposed to a fecal type of flora—the colon and terminal ileum.

2. The almost universal onset of CUC in the distal large bowel where antigen concentrations are likely to be greatest and mucosal contact most prolonged.
3. The proximal extension of CUC in contiguous fashion relating to the uniform presence of epithelial cell antigens and the possible that the skip areas of CD might be due to regional variations in the uptake of bacterial antigens.
4. The postsurgical recurrences of CD, particularly at anastomotic sites, which may reflect the establishment of a fecal type of flora at these sites as well as factors promoting the uptake of bacterial antigens.

The Shorter hypothesis represents an admirable attempt to synthesize the myriad of clinical and experimental observations concerning IBD into a plausible theoretical framework. Nevertheless, there are several important questions which are not fully answered by the hypothesis. First, in view of universal immunity to coliform antigens, why does IBD develop in relatively few individuals? Transient breaks in the intestinal mucosal barrier probably develop in most individuals, nor do all such breaks necessarily lead to IBD. More work is needed to elucidate the individual variations in immune responsiveness to colonic antigens. Also inexplicable are the occasional long periods of remission which punctuate the course of IBD. Such remissions might be due to some form of desensitization or tolerance induced by continued exposure to optimal antigenic concentrations, much akin to high zone or low zone tolerance observed in experimental situations. Alternatively, the selective suppression of lymphocyte-mediated immune reactions might be effected by a blocking effect exerted by antibodies directed against the same colonic antigens. Such a mechanism is supported by the observation the cytotoxicity of IBD lymphocytes for colonic epithelium may be blocked by pre-incubation of the colon cells with anti-colon antibodies (119). More work is needed to establish the interactions between cellular and humoral immune responses in IBD.

Conclusions

Given the currently available clinical and laboratory data it is still premature to conclude that IBD represents an autoimmune process. None of the six definitive criteria for autoimmune disease (See p. 602) have been well established for either CUC or CD. Nevertheless, although autoimmune mechanisms have not been conclusively established, there is a good deal of available data which supports an autoimmune etiology. Virtually all of the ancillary findings which Sell labels as presumptive evidence for autoimmune disease (See p. 603) have been demonstrated in IBD. Furthermore, it is possible to construct a theoretical framework, consistent with the available data, in which each of the Sell criteria is satisfied. Breakdown of colonic mucosal barriers might represent a *distinct immunizing event* in which the underlying enteric lymphatic tissue becomes exposed to coliform antigens. Following this immunization, a *latent period* might ensue during which time sensitized cells or antigen or both communicate with the systemic immune system, possibly via Peyer's patches. Clones of cells programmed to respond to the coliform antigen are produced, possibly in the thymus, and migrate to the lamina propria of the enteric tract. Upon *subsequent exposure* to coliform antigen or cross-reacting colonic antigens, these sensitized lymphocytes release lymphotoxin resulting in nonspecific local cytolysis. Damage to mucosal cells leads to the release of mucosal cell antigens and further compromises the mucosal barrier allowing more coliform bacteria to come into contact with lymphoid cells. The net result is a self-perpetuating reaction in which the inflammatory process leads to the release of those antigens which initiated the inflammation. These antigens, both bacterial and colonic, have been fairly well *identified* and their *chemical composition* is currently under analysis. The evidence for a transmissable agent may well represent a *transfer of the sensitive state by cells from an affected individual to a normal individual*, and the periods of remission which punctuate IBD may represent *the temporary induction of tolerance by optimal concentration of antigen.* Although these proposed mechanisms are purely speculative, they are useful in that they clearly point out those areas to which future research must be directed. Further characterization of the secretory and cellular immune systems in the gastrointestinal tract, as well as the interaction between the two, and of the immunogenetic factors regulating recognition of autologous tissue and response to antigens, seem to be the approaches most likely to shed light on a still enigmatic disease process.

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Watery Diarrhea, Hypokalemia and Hypochlorhydria in a 60-Year-Old Man: A Case Report

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Introduction

In 1958, Verner and Morrison (1) described a syndrome of refractory watery diarrhea and hypokalemia associated with non-beta cell islet tumors of the pancreas. Murray, Paton, and Pope reported a case of a non-beta cell islet tumor of the pancreas with water diarrhea, hypokalemia, and achlorhydria (2). It has recently been suspected that the cause of this syndrome is probably due to the secretion of the recently isolated polypeptide hormone Vasoactive Intestinal Peptide (VIP) (3, 4). This paper reports a case of watery diarrhea, hypokalemia and hypochlorhydria which was successfully treated by removal of a pancreatic non-beta cell islet tumor.

Case Report

A 60-year-old white male with a nine-year history of diabetes mellitus was admitted to The Mount Sinai Hospital with a chief complaint of weakness in his legs for three months and watery diarrhea for sixteen months.

Thirteen months prior to admission the patient had been admitted to another hospital for diarrhea. The diarrhea was described as watery brown, not mucoid and not greasy, but it did contain particles of undigested food. During that admission upper GI series and barium enema were within normal limits and a small bowel biopsy was reported as unsuccessful. The patient was discharged on Tetracycline, 250 mg, PO b.i.d. and potassium supplementation.

The patient continued to have diarrhea and was admitted to the same hospital one month before admission to Mount Sinai. At night the diarrhea was involuntary. There was never any pain associated with the diarrhea, but the patient did have occasional cramps. The diarrhea never contained blood. The patient never had melena or any formed stools since the onset of the diarrhea. The volume of diarrhea was approximately two liters per day divided into four to twelve bowel movements. Prior to the onset of diarrhea the patient had regular bowel movements and did not use laxatives. There is no history of decreased appetite, nausea, vomiting, ethanol abuse or weight loss. The patient has not travelled anywhere besides the Eastern United States and Western Europe.

On admission to the other hospital physical examination disclosed a thin, well-dressed white male, alert and in no acute distress. Pulse was 80 and regular, blood pressure 110/70, respirations 20 and temperature 37°C. The tongue showed decreased papillation with some erythema and dehydration. There was no thyromegaly. The rest of the physical examination was within normal limits.

Laboratory tests during that admission showed: hematocrit 46%, WBC 6400 with a normal differential. Admission SMA-6 showed glucose 228 mg/dl, BUN 20 mg/ml, Na 136 mEq/l, K 2.2 mEq/l, Cl 106 mEq/l, and CO_2 22 mEq/l. SMA-12 was Ca 9.5 mg/dl, phosphorus 2.4 mg/dl, uric

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acid 2.7 mg/dl, cholesterol 140 mg/dl, total protein 6.2 g/dl, albumin 3.8 g/dl, total bilirubin 0.5 mg/dl, alkaline phosphatase 225 mU/ml, LDH 140 ImU/ml, SGOT 35 mU/ml and creatinine 1.2 mg/dl. Additional lab studies showed normal values of serum lipase, amylase and cortisol. Urine analysis was within normal limits. Urinary 17-ketogenic steroids, 17-hydroxycorticoids, 5-HIAA and aldosterone were all normal. Histalogue stimulation test disclosed both basal and post-stimulatory decreased acid secretion. Serum T3, PBI and T4 were normal. Stool potassium was 150 mEq/l. Liver scan and IVP were within normal limits. Upper GI series with small bowel follow-through was within normal limits although the jejunum showed evidence of increased secretion. ECG showed a regular sinus rhythm with evidence of an old inferior wall MI.

The patient's potassium was corrected with intravenous fluids. The weakness improved. Tetracycline, Lomotil, pancreatic enzymes and 20 mg Prednisone daily did not help the diarrhea.

After four weeks the patient was transfered to Mt. Sinai for further studies. Sigmoidoscopy and barium enema were normal. Small bowel biopsy showed no significant changes in the jejunum. Colonoscopy was normal. Celiac angiography disclosed a five centimeter vascular mass probably in the distal pancreas. On the 23rd hospital day the patient underwent exploratory laparotomy. A 5-cm encapsulated tumor of the tail of the pancreas was found. There was no evidence of metastasis. A splenectomy and distal 60% pancreatectomy with removal of the tumor was performed. The pathology report indicated the tumor to be a large endocrine tumor probably of islet cell orgin. Special stains for carcinoid and beta cell granules were negative. It was not possible to tell whether it was of islet cell or enterochromaffin cell origin by light microscopy. The adjacent pancreas showed areas of hyalinization with accumulation of amyloid like material. No vascular of lymphatic invasion was found and the tumor was well circumscribed. Samples of the tumor as well as pre- and postoperative serum were sent to Dr. S. Said for VIP assay.

Postoperatively the patient was transferred to the ICU where he received intravenous alimentation for five days. On the eighth postoperative day the patient passed a soft formed stool.

Discussion

The investigation of diarrheogenic tumors have been intensely investigated since their first description by Verner and Morrison seventeen years ago (1). In 1961, Murray et al (2) suggested for the first time that achlorhydria may be a feature of the syndrome. In 1967, Marks et al (5) reported a case in which the diarrhea and achlorhydria were reversed by resection of the tumor and steroid therapy. They called this entity the WDHA syndrome. According to these authors, absolute achlorhydria is not consistently found with the syndrome and a low level of acid secretion may be compatible with its diagnosis.

In 1971, Barbezat and Grossman (6) described two peptides isolated from hog intestinal mucosa, vasoactive intestinal peptide (VIP) and gastric inhibitory peptide (GIP), which stimulate small intestinal secretion in conscious dogs. Glucagon and pentagastrin also stimulate, but secretin and the octapeptide of cholecystokinin do not. The effect of these peptides on gut secretion is similar to that of cyclic AMP (7).

VIP has been purified and sequenced by Bodanszky et al (8) and is found to be a polypeptide with 28 amino acids. Synthetic peptides corresponding to the C-terminal hendeca-, tetradeca-, pentadeca- and docosapeptide sequences showed activities which increased with increasing chain length. The C-terminal tetradecapeptides of VIP and secretin show considerable sequence homology. Known biological activities of VIP include vasodilation, lowering of arterial blood pressure, increase in cardiac output, enhancement of myocardial contractility, glycogenolysis, relaxation of tracheal, gastric and gall bladder smooth muscle as well as increased intestinal secretion (6–9).

Bloom et al (3) were the first to report the association of high preoperative plasma VIP level and high tumor VIP levels in the WDHA syndrome. In their series of six patients (five with tumors and one with islet cell hyperplasia) all had pathologically raised plasma VIP as compared to healthy controls and patients with laxative-induced diarrhea. Two tumors which were studied immunohistochemically both gave strong specific immunofluorescence with anti-VIP sera.

Additional evidence that VIP is the mediator of this syndrome is obtained from the work of Said and Faloona (4). They measured the plasma levels of VIP by radioimmunoassay in 25 controls and 30 patients with chronic watery diarrhea. Plasma levels were found to be too low to accurately measure in 22 of the controls and were significantly elevated in 26 of 28 plasma samples of patients and in each of 13 tissue extracts. Surgical resection of a pancreatic adenoma or successful medical treatment with prednisone or streptozotocin which was followed by a cessation of watery diarrhea was associated with a decrease in plasma concentration of VIP in the five patients able to be compared pre- and postoperatively. These authors report that of the patients examined thirteen had islet cell tumors and four had islet cell hyperplasia, but five had bronchogenic carcinoma, one pheochromocytoma and one ganglioneuroblastoma indicating that VIP may be the mediator of this syndrome which is caused by a variety of tumors.

It now appears that these "VIPomas" belong to a general class of polypeptide-secreting tumors known as "apudomas." These tumors consist of cells of the APUD (Amine content and/or Amine Precursor Uptake and Decarboxylation) series which are a group of cells which are thought to be derived from neural crest tissue and have several demonstrable characteristics in common:

1. Fluorogenic Amine content (eg., catecholamines)
2. Amine precursor uptake (eg., DOPA)
3. Amino acid decarboxylase

High content of:

4. Side chain carboxyl groups (masked metachromasia)
5. Nonspecific esterases and/or cholinesterase
6. α-glycerophosphate dehydrogenase
7. Specific immunohistochemistry for the polypeptide product of the cell.

Hormones produced by cells of the APUD system include ACTH/MSH, insulin, glucagon, gastin, calcitonin, enteroglucagon, secretin, GIP and VIP (10–12).

In addition to the surgical treatment of this disease, there is also a successful medical treatment that is useful when the tumor is inoperable or unable to be located. Streptozotocin, an inhibitor of pyridine nucleotide synthesis, is correlated with a selective toxicity to the pancreatic beta cell. In several patients described by Kahn et al (13) and Siegel and Muggia (14) there was a remarkable abatement of symptoms as well as decrease in plasma VIP level after a few weeks to months of treatment with streptozotocin.

Conclusion

The WDHA syndrome which has only been described in the past 20 years is well on its way to having its pathophysiology explained. There is now a method for assaying VIP which may lead to the early diagnosis and treatment of the syndrome by an alert physician.

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Ileorectal Anastomosis for Ulcerative Colitis

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Ulcerative colitis was first described as a separate disease entity by Willis and Moxan in 1875. The original therapeutic mainstay was irrigation of the colon and rectum with various drugs such as Dakin's solution. To facilitate such irrigation, Robson advocated in 1893 the establishment of an iliac colostomy with retention of the diseased colon and rectum which would, it was thought, improve with frequent irrigation. Cecostomy, appendicostomy, and loop ileostomy were subsequently proposed as more effective alternatives to colostomy, and in 1913 Brown, stressing that the main function of an ileostomy was to put the bowel to rest rather than to facilitate irrigation, proposed that a terminal ileostomy would be more efficient and easier to manage than the previously employed tangential ileostomy. Meanwhile, Lilienthal in 1901 performed a total colectomy with ileosigmoidostomy, but this procedure as well as the various ileostomies and colostomies failed to provide relief from the disease in the overwhelming majority of patients and the operation of choice became total proctocolectomy and the establishment of a terminal ileostomy (1–3).

However, total proctocolectomy has obvious, serious drawbacks from the patient's point of view. While undoubtedly curing the patient's ulcerative colitis it leaves him permanently handicapped by an artificial anus draining into a sac worn at his side. The psychological and physical handicaps of an ileostomy (restricted activities, danger of leakage, skin excoriation) have led many surgeons to search for a method of removing the colon and reestablishing intestinal continuity while eliminating the direct and indirect manifestations of ulcerative colitis. It was not until the years after the Second World War, however, that large enough numbers of patients were operated upon using intestinal continuity procedures to allow analysis of the results.

Ravitch (4, 5) in 1947 described a procedure of anal ileostomy in dogs. He tried it on two patients in 1948 and eventually had a series of 13 patients. The procedure consisted of stripping the anal mucosa, everting the anal serosa, pulling through the colon and ileum, and anastomosing, serosa to serosa, anus to ileum. His patients reportedly did quite well after an initially stormy postoperative period and suffered no incontinence or perianal excoriation several months after the procedure. Their stools were soft but formed, they had a modified but present defecatory urge described as a sense of fullness, and they could differentiate between flatus and feces.

However, Best (6) in 1952 reviewed the 12 cases of anal ileostomy available

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for followup that had been done by Ravitch, Wangensteen, and Best and found that 50% were failures, i.e., needed eventual ileostomies.

Meanwhile, Aylett in London was becoming champion of a similar but significantly different procedure. Since 1952 he has advocated the procedure of ileorectal anastomosis. By 1974 he had amassed a series of patients having undergone this operation that is much larger than any other series and his overall results will be considered later in this paper.

Schneider (7) in 1955 reported on four cases of what he termed ileoanal anastomosis. However, because of serious problems with perianal excoriation, leakage, stool looseness, urgency, and suture line breakdown in the first case, necessitating ileostomy, he subsequently preserved at least five centimeters of rectum. The immediate postoperative course of his subsequent three patients was also stormy, but progressed to near normalcy in weeks or months. Schneider also advocated the use of a protecting ileostomy until the suture line healed.

Mayo (8) reported in 1957 on 25 cases of ileorectal anastomosis. 22 were successful for at least several years after the procedure.

Turnbull (9) reported in 1959 on 13 cases in which he had followed Aylett's suggestion for a low rectal anastomosis. He had 10 clear successes and only one patient who needed a subsequent ileostomy.

A Ravitch-type anal ileostomy was tried by Cutait (10) on five patients. His report in 1960 showed poor results in all five and he advocated abandonment of the procedure.

In 1959 Abel (11), Corbett (12), and Muir (13), in separate series reported success rates of 10 out of 10, 16 out of 20, and 14 out of 15 respectively, using ileorectal anastomosis. Abel's patients also had been suffering from severe rectal stenosis due to ulcerative colitis and he had progressively and over a period of months dilated the rectum with a balloon prior to operation.

Goligher (14) reported successful ileorectal anastomosis in 14 out of 20 cases reported in 1961.

Griffin (3), working out of the University of Minnesota, reported one successful ileorectal anastomosis in a young woman done in 1942, and a series of 48 patients in 1963 who had undergone what he termed ileoproctostomies but were actually ileorectal anastomoses. He had good results in 25, fair results in 11, and two developed rectal cancer. Five anal ileostomies in the same series were all failures. Based on his series of ileorectal anastomoses Griffin recommended that very little ileum (less than one centimeter) should be removed and that the anastomosis should be low in the rectum.

Brown (12) reported in 1962 on 21 successes out of 27 patients receiving ileorectal anastomoses and pointed out that there were no deaths in the series and that two of the six "failures" were eligible for reanastomosis, i.e., nothing had been lost in any patient by attempting anastomosis. He emphasized that some rectal disease was acceptable but that he would not perform ileorectal anastomosis if there was destruction of the anal sphincter, severe disease of the anus, or severe disease of the rectum.

MacDermott (15) reported one successful anal ileostomy in 1964 and Oppolzer (16) proposed, the same year, a new procedure: He would evert the rectum, curette the mucosa, then reinvert the rectum; the mucosa was claimed to regenerate free from disease and was then anastomosed to terminal ileum. The three patients on whom he performed the procedure had normal sphincter control and two to three bowel movements per day. No one, however, has published subsequent reports of attempts at using such a procedure.

Wee (17) reported four successful ileosigmoid anastomoses out of four attempts. He found, in addition, that there was marked improvement in the condition of the rectum after removal of the diseased colon. However, Barker (18), in the same year (1963) reported only six successes out of 15 attempts and stressed the need for a protecting colostomy.

Foote (19) operated on 20 patients with ulcerative colitis in acute exacerbation and had a 50% success rate with ileorectal anastomosis. In addition, seven patients were classed as failures because of the frequency of their bowel movements but retained the anastomosis. Foote's contraindications to performing the procedure were cancer of the rectum, incontinent sphincter ani, and a markedly diseased rectum.

Out of 234 patients with ulcerative colitis seen between 1953 and 1966, Hughes (20) performed 63 ileorectal anastomoses. He claimed 48 successes with five deaths and ten patients needing subsequent (and easily performed) ileostomy. Among the successes there was no urgency, poor control, or perianal excoriation, even with mild persistence of rectal disease.

Ravitch (21), early champion of anal ileostomy, wrote later that ileorectal anastomosis was only indicated in patients with little or no rectal disease and that all of his patients having had such an operation had subsequently to be given an ileostomy.

Campbell (22) stressed the importance of a low rectal anastomosis in order to remove the upper rectum and rectosigmoid which were likely to become seriously diseased if left intact. An anastomosis at a low level would also allow easier viewing of the entire remaining rectum through a sigmoidoscope to detect possible malignant changes early. He gave as contraindications to ileorectal anastomosis the slightest defect in sphincter mechanism, gross hemorrhoidal disease, and submucosal or anorectal fistula (i.e., severe rectal disease). His 1965 series of seven patients had six successes and one conversion to ileostomy; no temporary ileostomies were performed.

Stating that ulcerative colitis always involves the rectum and that if there is no rectal involvement there is a misdiagnosis of ulcerative colitis, Maratka (23) emphasized that it is unrealistic to demand a healthy rectum as a prerequisite for ileorectal anastomosis and that a rectum-retaining anastomosis may be performed in all but the most severe cases. Using a staged procedure on 27 patients he had no failures, found improvement in rectal disease in most cases even though such disease persisted, and found no subsequent development of rectal carcinoma. An ileorectal anastomosis was only contraindicated if there was a lesion of the anal canal disturbing sphincter function, or perianal and perirectal fistulae, or advanced rectal stricturing. Usual hemorrhagic and ulcerative lesions of the rectum, moderate stenosis, or inflammatory polyposis were not contraindications.

Drobni, (24) a proponent of Ravitch's anal ileostomy, found good results with such a procedure in 31 out of 36 patients, with five postoperative deaths. There was no perianal excoriation after the immediate postoperative period. Drobni supported the Ravitch procedure because he claimed, without giving references or numbers, that "it is not uncommon that the disease of the bowel spreads from the rectal stump into the anastomosed small intestine" if ileorectal anastomosis is performed. This contention, however, is not supported by any other published report. Nor is Drobni's assertion that cancer develops "frequently" in the rectal stump.

An interesting survey was undertaken by Jagelman in 1969 (25). He interviewed 200 patients with ileorectal anastomoses and found them to be overwhelmingly in favor of such a procedure, taking into consideration their job performances, sex lives, and daily frequency of bowel movements.

Georgiadis (26) had eight successes out of 12 ileorectal anastomoses performed. However, two of his failures (who died) were operated upon while having an acute toxic megacolon, and he felt they would not have died had a temporary ileostomy been performed at the same time.

In a series of 27 patients in whom ileorectal anastomoses were performed between 1956 and 1968, Veidenheimer (27) found that 16 were clear successes, seven were acceptable, two were failures, and two died. He did not recommend using a protecting ileostomy and claimed that there was no difference if the procedure was performed in two stages (colectomy and ileostomy followed by ileorectal anastomosis) or one stage. However, he did point out that if the segment of rectum involved in the anastomosis is healthy, no failure will occur.

Baker (28) supports the view that ileorectal anastomosis for ulcerative colitis is an operation of above average mortality and morbidity, but that conclusion is not really supported by his own data. Of 39 patients who were operated upon and followed, Baker found 21 successes; and of the 21 only 13 had no complications at all. However, five of his "failures" refused to undergo ileostomy in spite of frequent diarrhea, two of the failures had the anastomosis performed at what Baker admits was too high a level, and of the nine procedures which were done in stages only one developed complications necessitating ileostomy. Significantly, there were no cases of malignant degeneration among the successes and there were no deaths at all in the series.

Sprechler (29) found 38 out of 48 patients in his 1971 series with successful ileorectal anastomosis. One of his failures was due to the development of rectal carcinoma, and there were six deaths. Sprechler refused to perform the procedure unless the rectum was soft and pliable and little or no disease was visible on sigmoidoscopy.

Adson (30) agrees with Sprechler that a prerequisite to ileorectal anastomosis is a normal or minimally involved rectum. In his 1972 series of 35 patients followed between five and 18 years he had 11 deaths overall (the same percentage, he points out, as for ileostomy over the same period of followup) with two deaths due to rectal carcinoma (in patients who had been extremely negligent in keeping their regular check-up appointments). He points out that young patients are most likely to need subsequent ileostomy, because of exacerbation of rectal disease.

Smith (31), citing his series of 25 patients with 19 successful ileorectal anastomoses over the years 1939–1972, feels that periodic proctitis after the procedure may be controlled with steroid instillations, and that the frequency of bowel movements may be kept low by retaining as much ileum as possible during the operation and by using hydrophilic muciloids postoperatively. He had no case of development of a rectal carcinoma but stresses the need for frequent sigmoidoscopic examinations as a prophylactic measure.

Nixon (32) performed ileorectal anastomoses on ten children with ulcerative colitis. The ten were among 19 children deemed suitable for surgery in a population of 80 affected children. All ten had relatively mild rectal involvement and all underwent a one-stage procedure since the author believes that if the rectum is so diseased as to warrant a two-stage procedure, a diverting ileostomy will not lead to enough rectal healing to allow the second stage of the procedure anyway. Of the ten only one needed rectal resection (for excerbation of symptoms) and that was not until five years after the procedure was performed.

By far the most extensive study of ileorectal anastomosis in ulcerative colitis has been done by S.O. Aylett in London (33). In his review of the 461 such procedures he has performed between 1953 and 1973 he points out that ileorectal anastomosis must be carefully differentiated from anal ileostomy in any discussion of the surgical treatment of ulcerative colitis. In his series he found only rare perianal excoriation, and, while the frequency of bowel movements may be up to six per day, they are well tolerated because there is no sense of urgency. He also states that while loss of potency never occurs in ileorectal anastomosis it is a not infrequent complication of proctocolectomy.

Aylett had 25 deaths in his series of 461 patients, a mortality rate of 5.5%, but 14 of the 25 were among the 54 cases of fulminating disease. 21 patients needed ileostomy and rectal resection because of rectal stricturing and increased rectal disease following the procedure. 12 patients developed rectal carcinoma and needed abdominoperineal resection; however, four of these patients were operated upon early in the series and would not have been considered suitable for ileorectal anastomosis in 1974, because of the severity of the rectal disease. There were no suicides in the series and among the 403 successes the average frequency of bowel movements was three to four per day, multiple pyodermia subsided, carbuncular abscesses cleared, arthritic changes were arrested, liver cirrhoses occasionally improved, and rectal disease largely subsided with excision of the colon.

Aylett recommends always using a temporary ileostomy because he found that 15% of the suture lines will leak for up to three months; in cases of fulminating disease the procedure should be done in two stages and in really acute cases a diverting ileostomy or transverse colostomy should be performed before the definitive procedure is attempted.

Ileorectal anastomosis is contraindicated if the patient, on first examination, has stricturing of the rectum, gross perirectal suppuration with fistula formation, or a patulous anus. It is fairly infrequent, however, for a patient to present with a condition that contraindicates the procedure since Aylett advised only 18 patients in the entire period not to undergo the procedure.

While Aylett's series clearly makes a very strong case for ileorectal anastomosis, Ritchie (34) had less dramatic results. From 1953 to 1973, 77 patients with ulcerative colitis had ileorectal anastomosis or ileosigmoid anastomosis or cecorectal anastomosis. There was one early and one related late death; two patients (who had both had ileosigmoid anastomoses) developed cancer of the rectum. Of 62 patients who were followed, 18 needed later rectal excisions for exacerbation of the disease; however, the success rate improved with experience and none of the 21 patients operated upon between 1969 and 1973 needed to be converted to an ileostomy. And it is of course relevant that Ritchie did not use one standard procedure, especially since Aylett has stressed the need for total colon excision and low rectal anastomosis.

A final very recent article is that by Fujiwara (35) who performed an anal ileostomy on a ten-year-old boy with ulcerative colitis with the principal lesions being in the rectum. The Ravitch procedure was used. Although the boy had severe diarrhea and perianal excoriation for about one month postoperatively, his stools became gradually formed and after 12 months he had near normal bowel function.

Conclusions

The conclusions to be drawn from the literature on ileorectal anastomosis for ulcerative colitis are fairly straightforward. There can be no question that the procedure usually works, i.e., provides relief from direct and related symptoms, and that it probably is not done often enough. The same cannot be said for anal ileostomy where the series are much smaller, the success rates lower, and the frequency of complications higher.

The indication for ileorectal anastomosis is simply ulcerative colitis for which resection of the colon is indicated due to severe, medically unresponsive disease. The contraindications should be limited to severe rectal disease, involvement of the anal sphincters, severe perianal fistulae, or severe anal involvement. In young persons, even though the long-term success rate may be lower, retention of the rectum for several additional years is of significant importance. It is also important to realize that while the procedure entails some risk, the risk is probably no greater than for colectomy and ileostomy, and the creation of an ileorectal anastomosis need not be the final procedure but can always be converted to a permanent ileostomy should the need arise at some later date.

While the success rates (ie., ileorectal anastomoses that do not need later conversion to ileostomy, and in which stool frequency is not a problem) vary from around 50% to around 100%, the various series presented would indicate that if Aylett's guidelines are followed the success rate with present day postoperative care would probably be closer to the higher figure. And the small minority of patients who receive an ileostomy subsequently have lost very little and will have retained intestinal continuity for at least some additional time.

The psychological consequences of permanent ileostomy are considerable in many cases, the stoma itself is not without problems, the operative and postoperative mortality of the two procedures is similar, and ileorectal anastomosis can always be easily converted to ileostomy, which is final.

While ileorectal anastomosis may not be suitable in cases of ulcerative colitis with severe rectal disease it would at least appear that it should be performed with much greater frequency than is now the case.

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Student Section

Insulinoma: Review of Seven Cases at The Mount Sinai Hospital in Light of the Current Literature

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Introduction

It has been nearly fifty years since Wilder (58) correlated endogenous hyperinsulinism with an islet cell tumor, seen during surgery on the pancreas. Two years later, in 1929, the first successful surgical excision of an insulinoma was recorded by Howland (18). After Whipple and Frantz (57) published their review of hyperinsulinism in 1935, Whipple firmly established association of three symptoms characteristic of insulinoma:

1. symptoms of hypoglycemia;
2. a blood glucose level less than 50 mg/100 ml;
3. relief of symptoms after ingestion of glucose.

Whipple's Triad has remained the fundamental approach to diagnosis of endogenous hyperinsulinism.

Since that time, there have been many reported cases and reviews on the subject of hyperinsulinism. The incidence of functioning islet-cell adenomas is uncommon; the discovery of these tumors at autopsy is reported to range between 1/8000 and 1/63 (59), but its incidence is more realistically estimated at less than 1/100,000 (43).

This study is of records of seven patients with insulinoma treated at The Mount Sinai Hospital since 1970; at least four of these cases were treated within the last year. A brief review of the current approach to diagnosis and management of this infrequently appearing entity is also attempted.

Clinical Data

Records of seven of the patients with insulinoma treated at Mount Sinai Hospital (New York) during the years 1970–1975, were available for the study. These patients included two males and five females averaging 47 years and ranging from 17 to 76 years of age.

Five of these patients were diagnosed as having benign insulinoma and two as presumptive insulinomas.

The symptoms with which these patients presented ranged in onset from eight months to seven years, averaging four years onset prior to admission. These symptoms included: disorientation (5)*, dizziness or altered gait (5), loss

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* Number in parenthesis after each symptom indicates how many patients had that symptom.

of consciousness (4), diaphoresis (3), unusual behavior (3), tremor (2), fatigue (2), hypoglycemic symptoms (2), weight gain (2), visual disturbance, palpitations, loss of memory, seizures, shortness of breath, unarousability, or drowsiness.

Diagnostic studies included: Fasting Blood Sugar (FBS), Immunoreactive Insulin (IRI), Glucose Tolerance Test (GTT), Tolbutamide Tolerance Test (TTT), Glucagon Provocation, Leucine Provocation, Calcium Infusion Test, Angiography, and Intraoperative Glucose Levels.

The value of FBS and GTT determinations were considerably enhanced by coupling these with simultaneous IRI levels; GTT was considered characteristically "flat" in three of the patients; TTT showed definitive insulinoma patterns in two patients. Both Glucagon and Leucine Provocation studies were diagnostic for organic hyperinsulinism in the two patients in which they were used. Celiac Axis and Superior Mesenteric Artery Angiography visualized tumor blushes in three of the four patients in whom adenomas were found surgically; one of these radiographic demonstrations was retrospective.

Treatment for four patients involved surgical excision of islet cell adenomas; two of these tumors were discrete nodules in the head of the pancreas and the other two lesions were located in the tail of the gland. One of these procedures required splenectomy with resection of the tail of the pancreas containing the adenoma.

Intraoperative blood glucose determinations were accomplished in two of the cases. Both displayed a rise in blood glucose to hyperglycemic levels immediately following excision of the nodules. A return to normoglycemia was spontaneously achieved in each case during the postoperative course.

Surgical excision was curative in the four patients mentioned. One patient was discharged on a regimen of Dilantin 100 mg q.i.d. Another with the diagnosis of presumptive insulinoma was discharged on a high carbohydrate diet; the second patient with this diagnosis was released without medications or treatment.

Case Reports

Case No. 1. H.E., a 44-year-old obese Dominican woman, had a two-year history of episodes of fatigue, dizziness, shortness of breath, diaphoresis, disorientation, and behavioral changes noted by family and friends. Starting six months prior to admission, episodes occurred most often between 4–6 p.m.

Physical examination was unremarkable. Laboratory examination was unremarkable except for a chest x-ray showing RUL scarring consistent with a history of tuberculosis thirty-five years prior to admission.

TTT was performed, provoking a drop in blood glucose from 65 mg % to 30 mg %, considered suggestive of insulinoma. FBS was 24 mg %. GTT was "flat" with a 2-hour blood glucose value of 28 mg %. Superior mesenteric artery angiogram displayed a 2.5 cm "blush" in the tail of the pancreas. Splenectomy was deemed technically necessary to accomplish resection of the tail of the pancreas, including a well-circumscribed 1.5 cm nodule. Immediately postoperatively, BS was 200 mg %, accompanied by transient glucosuria and acetonuria. By postoperative day 2, BS was in the 90–100 mg % range. Patient was discharged on postoperative day 17 without medication. Pathology reported a benign islet cell adenoma.

Case No. 2. S.R. was a 16-year-old white woman admitted for evaluation of a three- or four-year history of early morning profound weakness, relieved by eating. She had no history of syncope or

TABLE I

Summary of Presentations

Case No.	Patient	Age/ Sex	Onset of Symptoms	Symptoms	Angiography	Surgical Findings	Diagnosis	Treatment
1.	EH	44 F	2 yr	fatigue dizzy behavior changes disorientation shortness of breath diaphoresis	2.5 cm blush tail of pancreas	1.5 cm nodule (tail) splenectomy and resection of tail of pancreas	benign insulinoma	surgical excision
2.	SR	76 F	2 3 yr	"documented hypoglycemia"	unremarkable	—	insulinoma	Dilantin 100 mg q.i.d.
3.	LG	17 F	5 yr	syncope unarousable tremor disorientation drowsy unsteady gait	unremarkable	—	presumptive insulinoma	high CHO diet
4.	SR	56 F	5 yr	"hypoglycemia"	—	—	presumptive insulinoma	—
5.	DP	27 M	8 mo	weakness dizzy, diaphoresis syncope disorientation memory loss bizarre behavior weight gain 20 lbs/2 yrs	2 cm "dense stain" head of pancreas	2.5 × 0.7 × 1 cm brown well-circumscribed; head of pancreas	benign insulinoma benign inslinoma	surgical excision
6.	LS	56 F	7 yr	faintness palpitations visual blurring diaphoresis syncope seizure	unremarkable	2 cm well-circumscribed brown; tail of pancreas	benign insulinoma	surgical excision
7.	KS	54 M	6 yrs	syncope dizzy tremors disorientation behavior changes weight gain: 50 lb.	blush in head of pancreas	1.5-2 cm trilobar well-circumscribed head of pancreas	benign insulinoma	surgical excision

convulsions. Physical examination and Laboratory studies were unremarkable except for a sliding hiatus hernia detected by upper GI series and a blood glucose less than 50 mg %. Skull series was normal. GTT was performed showing values of 54 mg % glucose with 62 μ U IRI at T_0 and remained "flat" through 45 minutes (34 mg %/97 μU). After overnight fasting, blood IRI and glucose levels showed elevated blood insulin with marked fasting hypoglycemia. 50 % of subsequent FBS were less than 50 mg %. TTT performed before and after starting the patient on a course of Dilantin 100 mg q.i.d. showed, with medications, an improvement of both IRI/glucose ratios and symptoms. Celiac angiography failed to detect evidence of a tumor. Patient was discharged on Dilantin.

Case No. 3. L.G., a 17-year-old white woman was admitted complaining of episodes of stumbling and unsteady gait. Five years prior to admission, she twice lost consciousness after exercise. LMD reported her serum glucose to be low. One and a half years prior to admission, the patient was unarousable from sleep for three hours; upon awakening, the patient was disoriented and drowsy. During the year prior to admission the patient had several half-hour episodes of "drunken" gait, increasing in frequency. The patient also became drowsy in the middle of the day. Physical examination was unrevealing. Laboratory data were within normal limits except for blood glucose of 35 mg %. Subsequent tests included: FBS was 45 mg %; Leucine sensitivity test (Fig. 1); three hours after glucose loading there was a spinal fluid glucose of 54 mg % with concurrent 60 mg % blood glucose. Celiac angiography was unremarkable. Patient was discharged on a high carbohydrate diet ten days after admission with a diagnosis of hyperglycemia, presumptive islet cell tumor.

Case No. 4. S.R., a 56-year-old white woman was admitted with a five-year history of hypoglycemia manifested by confusion and speech slurring and relieved by ingestion of orange juice, though patient denied association of symptoms with fasting, anxiety, or exercise. Hypoglycemia was documented for several months by an HIP medical group, which had ruled out thyroid or adrenal dysfunction or malabsorption syndrome; also reported were random blood glucose levels of 25, 27, 74 and 36 mg % and a "flat" GTT. Physical examination was noncontributory. Laboratory data of significance included: a "flat" GTT to 180 minutes; basal insulin/glucose ratio of 64 μU/50 mg %; 28-hour fast showing glucose of 32 mg % with plasma insulin greater than 100 μU/ml; TTT causing a rise in plasma insulin to 99 μU/ml at 2 minutes, with blood glucose dropping from 78 mg % at the start of the test to 33 mg % at 60 minutes, and gradual return to baseline levels at three hours; glucagon provocation showed a rise in plasma insulin from a baseline of 58 μU/ml to 1,031 μU/ml by seven minutes. Since indication for celiac angiography and abdominal exploration was deemed contingent upon pending results of co-insulin and insulin antibody assays, the patient was discharged.

Case No. 5. D.P., a 27-year-old white man, complained of a one-year history of three episodes of leg weakness, syncope, disorientation, transient memory loss, and bizarre behavior, the latest of these attacks occurring ten days prior to admission. There was no history of seizures, headaches,

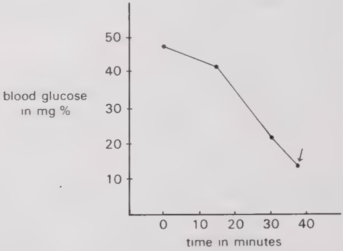

FIG. 1. *L-Leucine Sensitivity Test for L.G.* The test was stopped with an injection of glucose (i.v.) when patient became symptomatic at 38 min (arrow).

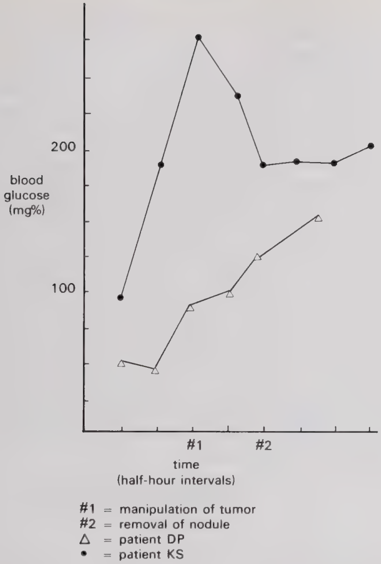

FIG. 2. Intraoperative blood glucose.

trauma, palpitations, diaphoresis, flushing, or diabetes mellitus. The patient had been treated psychiatrically during the past four years for "manifested severe anxiety neurosis." Physical examination was non-contributory except for two blood glucose values of 30 and 52 mg % around time of admission. GTT was unrevealing. Angiography demonstrated a densely stained area, approximately 2 cm in diameter, in the head of the pancreas; the study was evaluated as consistent with an islet cell tumor. During hospitalization, the patient displayed episodes of "acute hypermania" including running and doing somersaults down hospital corridors. The patient was explored surgically with excision of a 2½ × 1 × .7 cm well-circumscribed brown nodule, histologically, beta-cell adenoma. Intraoperative blood glucose levels were recorded (Fig. 2). The postoperative course was uneventful and after achieving normoglycemia, the patient was discharged.

Case No. 6. L.S. was a 56-year-old white woman with a seven-year history of loss of consciousness, palpitations, visual blurring and sweating, occurring about twenty times in the year prior to admission, including one such event relieved by orange juice. No pattern of associative factors could be established. She had no weight loss or generalized weakness. Physical examination revealed a BP of 200/110, treated with Hydrodiuril and Valium. Laboratory studies were unremarkable; Blood glucose was 78 mg %. Tests performed during hospitalization included: multiple oral GTT (Table II); 72 hour fast; a calcium infusion test (Table II); a glucagon provocation study (Table II); and an insulin tolerance test. Patient refused further Diazoxide after a three-day trial during which time patient experienced nausea and vomiting. Angiography failed to demonstrate a pancreatic lesion, though surgical exploration revealed a 2 cm yellow-brown, well-defined mass which was excised from the tail of the pancreas and subsequently defined histologically as benign islet cell adenoma. Immediately postoperatively a 300 mg % blood glucose level was detected; this decreased to the 110–150 mg % range several days before patient's discharge twelve days postoperatively on Hydrodiuril and Valium for control of essential hypertension.

Case No. 7. K.S. was admitted, a 54-year-old white man, with a 6-year history of hypoglycemia manifested by episodes of syncope, confusion lightheadedness, transient memory loss, tremors, incoordination of movements, and diaphoresis. After the initial syncope episode a diagnosis of left-sided cerebrovascular insufficiency was proposed and patient was treated with phenobarbital and 100 mg Dilantin q.i.d. Subsequently, patient reported no further syncopal attacks though the other symptomatology persisted. Patient found that he could relieve and prevent attacks by ingestion of orange juice and eating more frequently, result of which was a 50-pound weight gain. Patient noted increasing frequency of attacks; and fasting for a GTT performed by his private physician caused an episode of loss of consciousness. Results are recorded in (Fig. 3). An IRI level of 17 μU/ml with a 38 mg % blood sugar was recorded. Admission examination revealed an obese, diaphoretic man with cool skin. Significant laboratory values included fasting blood sugars of 26 and 14 with random samples consistently below 45 mg %. An IRI of 34 μU/ml was determined with blood glucose of 48 mg %. Patient refused a TTT. Angiography displayed a blush interpreted initially as duodenum. Surgical exploration exposed a 2 cm trilobular tumor in the head of the pancreas, corresponding with the localization of the angiographic blush. The mass was excised and pathology investigation revealed a Beta-cell adenoma. Multiple blood samples for glucose and insulin levels were taken intraoperatively (IRI levels pending, glucose Fig. 2). Postoperatively, blood sugar dropped from 180 mg % to values around 90 mg %. A GTT on the third day postoperative is recorded (Fig. 3). Patient was asymptomatic during fasting and was discharged two weeks postoperatively.

TABLE II
Data for Patient LS

Time	GTT #1	GTT #2	TTT	Glucagon	Calcium	Insulin Supp.
0	66/36	60/29	68/14	60/10	68/24	67/2.26
1				72/233	68/14	
2			66/217			
3				77/776	69/17	
4						
5			64/1110	81/789	70/18	
10				85/900	67/20	69/1.74
15	78/461	62/134	42/1352	72/859	68/20	
20				65/611	66/18	73/1.58
25				53/283	65/11	
30	52/958	53/737	12/380	55/319	65/12	55/1.54
45	32/280	56/929	14/94			38/0.95*
50						24/1.13
60	34/141	51/464	24/58	20/43	65/12	26/1.35
90	51/56	55/88	29/29		67/14	
120	46/37	68/99	39/20		64/22	
180	63/230	55/39	51/18		63/12	
240	49/48	59/10				
300	65/33	56/8				

* Sampled at 40 min.

Above values are expressed as a ratio: (glucose mg %)/(IRI μU/ml), except insulin suppression data, which is (glucose mg %)/(C-peptide ng/ml).

Above data provided by courtesy of Elliot Rayfield, M.D.

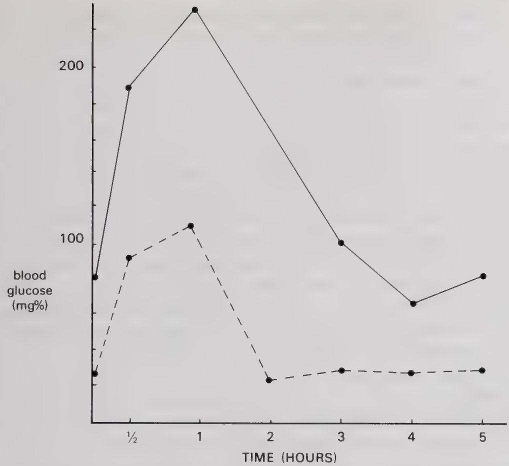

FIG. 3. Glucose tolerance test of KS. Dashed line represents GTT at time of admission and solid line represents GTT performed postoperatively.

History and Presentation

The diagnosis of insulinoma is contingent upon a high index of suspicion. Freeark and DePeyster (23) extended the widely known Whipple's Triad to suggest that investigation for organic hyperinsulinism should be stimulated by a history of: 1) disturbances of consciousness; 2) increased appetite with rapid weight gain; 3) paroxysmal behavior disorders and/or transient neurologic deficits; 4) acute "alcoholic" behavior without ethanol ingestion.

A study of 193 patients with insulinoma (59) showed the symptomatology most often encountered (Table III). Occurrence of episodes is characteristically exacerbated by protracted fasting or exercise. The patient will often discover that relief or avoidance of symptoms can be achieved by more frequent eating, resulting in weight gain.

Hyperinsulinism secondary to beta cell tumors is clinically manifested most commonly in the 30- to 60-year-old age group (59); diagnosis of insulinoma is rare in neonates (55, 59) and adolescents (55).

Pathophysiology

The essential pathophysiology of insulinoma is an insulin level inappropriately elevated with respect to blood sugar. Insulin secretion is normally diminished or inhibited in the presence of hypoglycemia, which stimulates glucagon-induced hepatic gluconeogenesis. An insulinoma is relatively insensitive to hypoglycemia and continues to maintain a relative hyperinsulinemia, which also abolishes liver gluconeogenesis, even in the face of the hypoglycemia.

The source of hyperinsulinism is most often (70%) a single benign tumor within the pancreas (39); between 4–12% (6, 14, 27, 46, 50) of the cases reveal multiple adenomas; another 10% are considered malignant. The size of the tumor may range from microscopic (14) to a mass over 1500 gm (39) though the majority are in the 10–50 mm range (59). Some workers (27, 59) have found distribution within the pancreas to be about equal among head, body, and tail regions of the organ, while LaRoche (25) found 42% in the body alone. About 2–5% (6, 27) insulinomas are found ectopically.

Color of the well-encapsulated mass varies from white to yellow-brown to purple. The histology is most often akin to normal islets of Langerhans morphology (37, 39). Priestly (37) claims that the malignant varieties of insulinoma are evident histologically, while Howard (39) indicates that there may be a considerable discrepancy between the morphologic and clinical behavior of these neoplasms.

It has been reported (4, 11) that cells of insulinoma contain less insulin than normal Beta cells, and that the major functional abnormality associated with these cells may be a defective storage capacity. This is supported by the findings of Creutzfeldt et al (4) that ultrastructurally atypical or absent Beta granules are found in insulinoma cells. Furthermore there is a significantly higher percentage of proinsulin-like components (PLC) circulating in patients with insulinoma (4); this information is a basis for the evaluation of pancreatic function by C-peptide analysis (42).

TABLE III

Signs and Symptoms (In Order of Decreasing Frequency) (59)

Loss of consciousness
Confused state
Weakness and fatigue
Deep coma
Sweating
Drowsiness and stupor
Visual disturbances
Amnesia
Clonic convulsions
Noisy behavior
Headache
Tremor
Hunger
Positive Babinski sign
Paresthesias
Irritability
Transient hemiplegia
Abdominal pain
Palpitation

Wermer (39) noted the occurrence of adenomas of other endocrine glands, primarily pituitary and parathyroids, simultaneously with islet cell tumors. This Multiple Endocrine Adenomatosis may be seen in close to 10% (27) of patients presenting with hyperinsulinism. This phenomenon has a familial proclivity (14, 59).

The clinical manifestations of the insulinoma are a direct consequence of the degree of relative hyperinsulinism. As mentioned previously the actions of insulin in producing hypoglycemia are multiple: peripheral tissue take-up of glucose and inhibition of glucagon, in addition to the direct effect of insulin on hepatocytes, result in decreased gluconeogenesis, decreased hepatic glucose output, and decreased circulating glucose. Of consequence also is the rate at which glucopenia occurs. When gradual decline in glucose levels occur, the milder symptoms of hyperglycemia are manifested (hunger, irritability, light-headedness, weakness and fatigue). If the drop in blood glucose is unchecked the patient may experience syncope. In these instances the slow progression of the hyperglycemia may result in catecholamine release being inconsequential to the symptomatology (59). However, when the glucopenia is rapid in its development, the patient may display tremors, palpitations, and convulsions. The long-term effect of a chronic hyperinsulinism is manifested most often as a deterioration of intellect, often attributed to a "primary" psychiatric etiology.

The clinical history and presentation of the patient with insulinoma may provide clues as to the benign or malignant nature: the less severe, chronic picture is more typical among patients with benign pathology; the severe symptomatology of more recent onset is suggestive of a malignant insulinoma. However, the clinical pictures may be indistinguishable (59) or even misleading (17, 39).

Differential Diagnosis

For diagnosis to be possible the symptoms of the patient must suggest a hypoglycemic etiology (see Table IV). The clue to the identification of insulinoma is recognition of the characteristic features mentioned earlier. The hallmark of insulinoma is the finding of inappropriately high insulin levels with hypoglycemia. There are a number of tests which can be performed to eliminate other etiologies and confirm the diagnosis.

Fasting Blood Sugars. A 15-hour overnight fast should produce hypoglycemia at some time in 80% of insulinoma patients and in nearly 100% of patients subjected to frequent sampling on different days (28).

Of itself, a fasting blood sugar less than 50 mg % is considered only presumptive evidence (7), establishing the presence of hypoglycemia but not the etiology. This test is of much more significance when frequent random blood samples are assayed with simultaneous insulin levels.

Immunoreactive Insulin. Since Yalow and Berson (61) described the immunoreactive insulin assay, this test has been an essential element of the laboratory diagnosis of insulinoma. Marks (27) claims more than 95% of

TABLE IV

Etiologies of Hypoglycemias (59)

- *Deficient food supply*
  - Starvation
  - Excess loss from body
  - Hyperutilization (excercise, fever, neoplasms)
- *Hepatic disorders*
  - General hypofunction (circulatory deficiency, cirrhosis, hepatitis)
  - Glycogen storage disease
  - Galactosemia
  - Hereditary fructose intolerance
  - Familial galactose and fructose intolerance
  - Other rare hereditary disorders
  - Ethanolism
- *Deficiency of insulin-counteracting hormones*
  - Growth hormones
  - Glucosteroids
  - Catecholamines
  - Glucagon
  - Thyroid hormones
- *Disorders with abnormal secretion of insulin*
  - Tachyalimentation (posthyperhyperglycemia)
  - Posthyperglycemia (functional)
  - Early diabetes
  - Hypoglycemia in newborn infants of diabetic mothers
  - Idiopathic familial
  - Insulinoma
- *Non-Beta cell neoplasms*
- *Nervous system disorders*
- *Pharmacogenic*
  - Excess insulin (iatrogenic or factitious)
  - Sulfonylureas
  - Other agents

insulinoma patients will show relative hyperinsulinemia, but also notes that multiple sampling on different days is often necessary to demonstrate relative or absolute hyperinsulinism. Marks and Samols (28) propose that the high levels of insulin may exist in the hepatic portal venous blood without being reflected in the peripheral blood. Alternatively, or conjointly, they add that benign insulinomas tend to release spurts of the hormone, rather than a continuous increased insulin secretion; since insulin has a short half-life (about 35 minutes), the peaks are of short duration (62) and may be missed by isolated or infrequent sampling techniques (28).

The IRI assay provides critical information when provocative tests are done.

Proinsulin. The utility of this assay has already been discussed in the context of its histopathological basis.

Prolonged Fast. An attempt to precipitate neuroglycopenic symptoms may employ a 72-hour fast with exercise. The diagnostic value of this relatively costly and uncomfortable test is limited: other causes of hypoglycemia (alcohol-induced, obesity, idiopathic hypoglycemia of childhood) may respond in a manner similar to that of insulinoma. The tolbutamide, leucine and glucagon provocative tests may be of considerably more utility with less discomfort (28).

Glucose Tolerance Test. Many different responses have been noted when patients with insulinoma have been subjected to the oral or intravenous glucose tolerance test. Most characteristic of the patient with hyperinsulinism will be the "flat" curve, displaying unusually low values throughout the test; this result is secondary to the rapid sequestration of the infused glucose into peripheral tissues in the presence of inordinately high circulating insulin levels. The transient rise in blood glucose secondary to loading at the start of the test may stimulate an even further rise in immunoreactive insulin levels, with a resulting "overshoot" to glucose levels lower than the pretest baseline, so that if the overnight fast does not cause symptomatic hypoglycemia, a reactive hypoglycemia may produce symptomatology. For this reason a glucose infusion should be ready to end the test if signs of glucopenia develop.

Tolbutamide Tolerance Test. One gram of tolbutamide in 20 ml normal saline is infused over a 2-minute period in the fasting patient. Samples of blood for simultaneous immunoreactive insulin and glucose levels are taken before infusion, at 5, 10, 15, 30, 45, 60, 90, 120, and 150 minutes after infusion. About 80% (28) of patients with insulinoma will show a normal or exaggerated fall in blood glucose (greater than 50 mg %) which may be sustained the duration of the test. Within five minutes the plasma insulin will achieve values exceeding 150 μU/ml, (and may rise above 1000 μU/ml[6]), returning to preinfusion levels more gradually than in normal patients. The most potent stimulus of insulin release in patients with insulinoma (11), tolbutamide, can induce severe glucopenia, demanding preparation to infuse a glucose solution if symptoms warrant termination of the test.

Steinke and Soeldner (51) recommend use of tolbutamide to reduce time needed for producing symptomatic hypoglycemia in patients with insulinoma; a 24-hour fast with 1 gm of tolbutamide will suffice in patients that may otherwise require up to 72 hours before becoming symptomatic.

Conversely, tolbutamide should not be administered to patients who are hypoglycemic at the time of the test.

Glucagon. One mg glucagon is rapidly administered intravenously, producing an exaggerated rise of insulin levels within 5–10 minutes in 70% of patients with insulinoma (27). Because the glucagon stimulates insulin production and hepatic release of glucose, the glucagon test (also 1 mg) will produce a modest rise, primarily in arterial blood, within two hours, to be followed by an exaggerated symptomatic reactive hypoglycemia in 80% (28) insulinoma patients. If samples are drawn every five minutes for the first ½ hour and then every 10 minutes for the second ½ hour, immunoreactive insulin peak values greater than 150 μU/ml can be expected in nearly all (24, 29) patients with insulinoma as compared with immunoreactive insulin values less than 100 μU/ml in normal patients, or patients with reactive hypoglycemia (28). Thus the glucagon provocation test is more specific for insulinoma than the TTT.

The Glucagon Test is strongly recommended by Kumar et al (24) because it is a much safer test than the TTT, which can produce a more severe hypoglycemia. Kumar also notes that patients on drugs known to influence insulin release (diphenylhydantoin, hydrochlorothiazide, tolbutamide, and aminophylline) should discontinue their use for several weeks prior to testing.

Leucine. In 1959, Schwartz and coworkers (45) introduced an L-leucine stimulation test to aid in diagnosis of insulinoma. Between one-third (28) and three-fourths (45) of patients with insulinoma will experience symptomatic hypoglycemia and elevated immunoreactive insulin levels within 20–45 minutes after oral ingestion of L-leucine in doses of 150–200 mg/Kg bodyweight.

Floyd et al (11) found seven of eight insulinoma patients to show immunoreactive insulin values higher than those seen in normal patients (range 0–28 μU/ml); the mean immunoreactive insulin values for these eight insulinoma patients was 211 μU/ml with a range of 13–808, with sampling approximately every 10 minutes for at least 30 minutes. These workers point out that the degree of neither the hypoglycemia nor the hyperinsulinemia is as profound as in the tolbutamide test, and the overlap between values of normal patients and of patients with insulinoma require demonstration of immunoreactive insulin values greater than 30 μU/ml in diagnosing the insulinoma, but values less than this do not rule out the pathology. Furthermore, children with leucine-sensitive idiopathic hypoglycemia of infancy and childhood will also demonstrate excessive hyperinsulinemia with an oral dose of this amino acid (61). Therefore, this phenomenon must be differentiated from insulinoma in children by other provocative tests.

Insulin Suppression. Turner and coworkers (52–54) have reported the efficacy of employing fish insulin to suppress normal endogenous insulin secretion. Their method involves an overnight fast followed by 1 ml injection of 0.2 units of fish insulin every 45 minutes with subsequent blood sampling each 10 minutes, stopping the test with intravenous glucose solution when hypoglycemic symptoms appear. The endogenous solution levels can be measured by assay using antisera with greater affinity for human insulin than the fish insulin.

These workers feel that a suppression test is a more direct, dynamic, and sensitive method to reveal insulinoma by its lack of normal physiologic response to high circulating insulin levels and hypoglycemia. The study of Turner and Harris (52) on fish insulin suppression showed insulin levels consistently higher in twenty patients with insulinoma than in the twelve normal subjects.

Horwitz and Rubinstein (16) showed similar ability to distinguish a patient with insulinoma from several subjects by using porcine insulin (Fig. 4) infused at a rate of 0.1 unit/Kg bodyweight/hour, continuing for one hour or until symptoms of hypoglycemia are manifest. Since porcine insulin will cross-react in the human insulin immunoassay, these investigations make use of assay for C-peptide (serum proinsulin connecting peptide) to assess endogenous insulin levels.

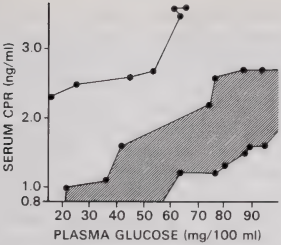

FIG. 4. Insulin suppression. Relationship of C-peptide immunoreactivity (C.P.R.) to plasma-glucose during insulin infusion in 7 normal subjects (stippled area) and a patient with insulinoma (from Horwitz and Rubenstein, ref. 16).

Radiology. Most reporters (6, 12, 14, 21, 23, 27, 37, 43) agree that the role of radiology in evaluating and treating hyperinsulinism is angiographic localization of a tumor mass after insulinoma has been investigated by biochemical means. Preoperative localization by celiac axis angiography producing the characteristic "tumor blush" of an insulinoma will aid in planning surgery and in reducing the operative time, surgical manipulation, and likelihood of overlooking multiple or ectopic lesions. Prior to the adaptation of this technique to localization of insulinomata by Olsson (35) in 1963, tumors were found only by intraoperative gross observation of a nodule or by palpation of a mass within the pancreas after mobilization of the organ. Because islet cell tumors average 1 to 2 cm (7) with only one-fourth of the tumors exceeding 3 cm (30) intraoperative localization can be difficult or impossible. Rösch (41) claims that an insulinoma of 5–10 mm or greater will be reliably detected angiographically. However, experience (2, 9, 23, 31, 40) shows a wide range (38–100 %) of successful arteriographic localization. This disparity among reporters may be attributed to: 1) bias introduced by tendency to report successes; 2) whether localization is achieved with a high degree of confidence pre-operatively or the identification is retrospective; 3) higher frequency of localization of larger-sized tumors; 4) degree of tumor vascularity; 5) location of lesion within the pancreas; and 6) whether superselective techniques (vasoconstrictive pharmacoangiography) are used. The excellent study of Robin et al (40) considers 60–70 % successful preoperative localization to be a good estimate. Some investigators (31, 40) recommend demonstrating the tumor in at least two projections to enhance the three-dimensional placement of the mass and further contribute to planning expeditious surgery.

McKinnon et al (32) advocate gastric insufflation to aid differentiation of gastric and duodenal vessels from those of the pancreas.

Angiography can also be of utility in predicting whether the lesion is benign or malignant or if metastases are present (9, 13, 31, 32). Benign tumors tend to appear as round and discrete foci, whereas the malignant lesions often show irregular contours, ill-defined borders, and tortuous vasculature (13, 31). Metastases most often appear in peripancreatic lymph nodes or liver (32).

The degree of false-negative or false-positive reports varies with factors mentioned previously in connection with successful arteriographic localization (as well as the skill of the radiologist).

The technique of celiac and superior mesenteric angiography is considered safe, complications being nonexistent or negligible (27, 31), and is indicated for all patients in whom the diagnosis of insulinoma has been established.

The techniques of tomography (27) or echography (23) are not considered efficacious for evaluation of insulinoma. ^{75}Se-selenomethionine has been used for radioactive isotope scanning of the pancreas in patients with insulinoma (15, 60) but scintiscans are expensive, less sensitive, less precise, and difficult to interpret (6).

Calcium Infusion. Calcium ion has already been linked to normal physiologic insulin release (5, 32) and patients with primary hyperparathyroidism show statistical fasting hyperinsulinism compared with postparathyroidectomy, normocalcemic insulin levels. Kaplan and coworkers (20) have shown that normal volunteers show no significant change in circulating insulin/glucose or insulin levels after calcium-infusion-induced acute hypercalcemia.

However, these researchers also found that within half an hour, infusion of 4 mg Ca^{++}/Kg/hour (calcium gluconate or calcium chloride, intravenously) into a patient with insulinoma caused a threefold rise in serum insulin (to greater than 150 μU/ml) with a drop of serum glucose from 50 to 26 mg %. After enucleation of the tumor, the patient's response was similar to that of the group of normal patients.

On this basis, it has been suggested (20, 36) that provocation by calcium infusion may be found useful in diagnosing insulinoma.

Intraoperative Identification of Insulinomas

A description of the operative technique in a patient with insulinoma will not be discussed in this paper. However, it is emphasized that mobilization of the pancreas is critical even when a discrete nodule is readily apparent. A meticulous visual and digital examination of the entire substance of the pancreas is mandatory to obviate subsequent operation for other insulinomas in patients with multiple adenomas.

When no tumor is detectable after a diligent search the surgeon should inspect the following areas (in order of decreasing frequency) for an ectopic focus (6): stomach, duodenum, jejunum, ileum, Meckel's diverticulum, behind the portal vein, in the omentum, and in the splenic hilum.

In the event no tumor is demonstrated, different degrees of subtotal distal pancreatectomy are recommended (6, 14, 23, 25, 50). Few prefer pancreatic-duodenectomy (Whipple procedure); this approach carries a higher mortality than the more conservative resection (6). Cure rate is proportional to the amount of organ resected (6), since there is uniform distribution of the adenomas throughout the pancreas.

Keaveny and his coworkers (22) reported a case of islet cell tumor with two metastatic lymph nodes located intraoperatively. Injection of one percent toluidine blue-0 into the splenic artery resulted in almost immediate staining of only the areas containing tumor.

Intraoperative measures of blood sugar have been reported by many investigators (6, 14, 19, 22, 23, 25, 44). It is concluded (6, 14) that values have been misleading if employed to determine whether the entire insulinoma has been resected. Even in cases of retained hyperfunctioning pancreatic islets requiring subsequent therapy for hyperinsulinemia, intraoperative elevations of blood glucose similar to the patterns in patients cured by surgery were seen (14). Harrison et al (14) also report a case where no significant intraoperative elevations of blood glucose were seen after curative removal of the single adenomas.

Measuring intraoperative blood glucose may show the characteristic immediate alterations after surgical removal of the hyperfunctioning tissue, yet this assay must not be considered as definitive evidence of cure.

Medical Management

Treating insulinoma medically is indicated when: (1) surgery is not desired by the patient; (2) the lesion is not resectable due to malignancy; (3) the patient is a poor surgical risk; (4) the morbidity and mortality is deemed too high or disproportionate to the patient's symptoms (eg. Whipple procedure after a non-curative sub-total pancreatectomy); (5) the patient is to be maintained until the time of operation.

Palliative

Diet. Patients have often learned to eat more frequently during the day and night; ingestion of food (especially orange juice) relieves glucopenic symptomatology.

Anti-hormonal. Diazoxide (300–800 mg/day or 10–15 mg/Kg/day), an anti-diuretic benzothiadiazine, does not affect hormone synthesis, rather it directly inhibits insulin release (4, 43). Diazoxide also stimulates epinephrine release, with its affects increasing blood glucose (43, 59). Side effects include fluid retention, gastrointestinal irritation, hypertrichosis, and agranulocytosis (59).

Diphenylhydantoin. Dilantin helps to raise blood glucose levels by inhibition of insulin release both from labile and storage pools (whereas diazoxide blocks only the former) (26).

L-asparaginase was used in a patient with malignant insulinoma (43) refractory to streptozotocin. Subsequently, increases in blood glucose were seen, but therapy was discontinued because of hepatotoxicity. The drop in plasma insulin was attributed to decreased insulin synthesis. Schein (43) recommends that this agent may be useful for short-term palliation.

Glucocorticoids. Glucocorticoids produce undesirable side effects, prohibiting their use with insulinoma patients.

Anticancer Agents.

Using the cancer chemotherapeutic agents *5-fluorouracil*, *Alloxan*, and *Nitrogen Mustard* to treat metastatic islet cell carcinoma has shown poor results because of toxicity to host or lack of tumor response.

Streptozotocin. N-methyl-nitroso-urea-glucosamine is derived from Streptomyces achromogenes, and displays a cytotoxic effect specifically on beta cells (23, 43). Relief of symptomatology and reduction of tumor mass are achieved with its use (43). Drug toxicity includes renal tubular damage manifested by proteinuria, nephrogenic diabetes insipidus, and azotemia (43).

Summary

There are many techniques available for confirming the suspicion of insulinoma in patients who present a suggestive history. Selection of the appropriate tests is guided by the presentation of the patient and the facilities available to the investigator.

From the foregoing discussion it may be concluded that use of the immunoreactive insulin assay is the major factor in most of the significant studies; this logically follows from the nature of the pathology, a hypersecretion of insulin, either absolute or relative to blood glucose levels.

Generally, results of fasting blood sugar determinations or glucose tolerance tests are too variable (28) and nonspecific to be of any significant value in diagnosis of insulinoma. However, the record of K.S. (Case No. 7) exemplifies a situation in which results of these tests are so characteristic as to be diagnostic, especially when simultaneous IRI levels provide direct evidence of the pathology.

In most other cases, including the six other patients reported here, the clinician will need to look to more reliable studies. Prolonged fasting with exercise is both uncomfortable and costly (since the patient must be hospitalized during the fast) and the information provided by this test, can be more readily and inexpensively gleaned by a shorter overnight (14–15 hours) fast coupled with a dose of tolbutamide, glucagon, or leucine (28). Even so, more information is available when these substances are used for their respective provocative tests.

In normal patients, the most potent stimulus for insulin release is glucose, followed in order by tolbutamide and leucine. In patients with insulinoma the order of potency is tolbutamide > leucine > glucose (11).

The tolbutamide tolerance test usually produces the most profound rise in blood insulin levels among patients with insulinoma. Khurana et al (21) dispute the finding of Steinke and Soeldner (51) of diagnostic hypoglycemia with hyperinsulinemia in 90% of insulinoma patients; however, the higher success rates are achieved when more frequent sampling is done during the first fifteen minutes after tolbutamide infusion. Since insulinomas release spurts of insulin (28), which has a short half-life in blood (62), the transient peaks of insulin levels after stimulation may be missed by infrequent blood sampling. Kumar et al (24) recommend blood sampling every five minutes during the first half-hour of a glucagon stimulation test.

Though glucagon-induced hyperinsulinemia (21) and leucine-induced hypoglycemia (21) are more sensitive (fewer false-negatives) in detecting insulinoma, leucine is less specific, producing hyperglycemia in patients with leucine hypersensitivity (14, 21).

In five of the patients reported in this study, one or more of these provocation tests were employed and aided in establishing the diagnosis.

Relatively few investigators have reported use of calcium infusion (20, 32, 36) or insulin suppression (16, 52–54) studies in diagnosing insulinoma. Both of these methods were employed in one of the currently reported patients; the obtained results were not typical for insulinoma, and the patient was subsequently found to have a "nonautonomous insulinoma."

Many reports (6, 12, 14, 21, 23, 27, 43) including this study have found celiac axis angiography to be of utility primarily for preoperative localization of the lesion after documentation of insulinoma by other means.

Intraoperative blood glucose determinations were recorded in two cases reported here. Though a rise in blood glucose is seen immediately following excision of the tumors, it is noted that postexcision hyperglycemia is considered neither necessary nor sufficient evidence of successful surgical treatment of insulinoma (6, 14).

Acknowledgments

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Student Section

Surgical Implications of Schistosoma Mansoni Infestation

RENÊ B. WILSON, B.A.

Introduction and Description of the Parasite

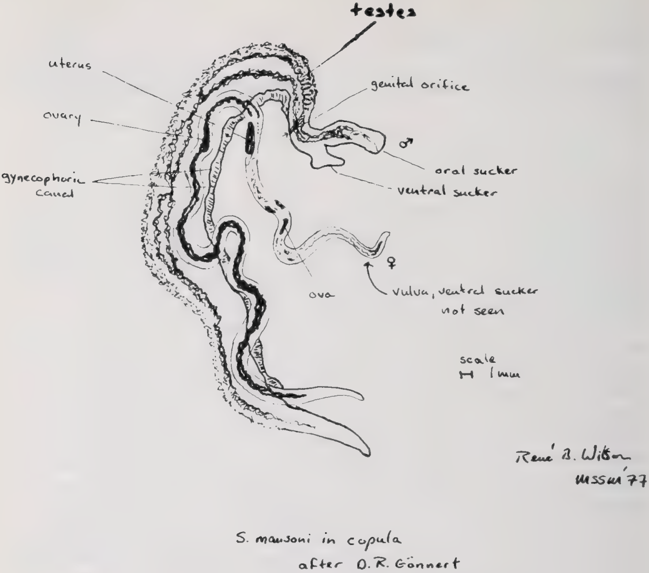
Schistosoma mansoni is a digenetic trematode worm that has a complex life cycle. Man is the definitive host of S. mansoni as well as the other members of the genus Schistosomatoidea: S. haematobium and S. japonicum. Worldwide schistosomiasis is the most frequent cause of cirrhosis of the liver, and there were about 200 million people affected with the disease in 1969.

In 1851 Theodor Bilharz discovered S. haematobium in the mesenteric venules of an Egyptian farmer at Cairo, but it was not until 1901 that studies of somatic and egg morphology and host parasite relationships allowed separation of the two species of occidental schistosomes from each other. Schistosoma haematobium chooses the urinary bladder as the exit from the body, whereas S. mansoni ova most often pass in the stool after transmucosal erosion at the distal colon. The ova of the two species are differentiated on the basis of the spine, S. haematobium having a terminal spine and S. mansoni a lateral one. Differences in body morphology between the two species are slight, and as they often coexist in the same host in Africa, it is easy to understand the initial confusion as to their identity. Although man is the definitive host, rodents may be infected and perhaps in some instances constitute animal reservoirs of the disease.

Manson was the first to describe laterally spined eggs on the American continent in an African living in Antigua. The first native American to be described with the disease was reported from Puerto Rico by Gonzales-Martinez in 1904. The parasite is indigenous to Brazil and Venezuela in addition to Africa, the Mideast, and the Caribbean.

Schistosoma mansoni adult male and female body morphology is shown in Figure 1. Male worms differ from the other two species by the more prominent tubercles on the body. Color is white, and body size averages 9 mm × 2 mm in the male and 12 mm × 1 mm in the female. The male has 6 to 9 testes which empty by a short duct near the anterior end of the gynecophoric canal. The female's uterus is as shown, and the genital tract opens near the mouth. The uterus contains but 1 to 2 eggs at a time in contrast to the other two species, but up to 300 ova per day may be produced.

From Mount Sinai School of Medicine of The City University of New York, N.Y. 10029, submitted in partial fulfillment of the Third-Year Clerkships requirement for the Department of Surgery. Mr. Wilson is currently a fourth-year medical student.


testes
uterus
genital orifice
ovary
♂
oral sucker
gynecophoric canal
ventral sucker
♀
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vulva, ventral sucker not seen
scale
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René B. Wilson
mssm '77
S. mansoni in copula
after D.R. Gönnert

FIG. 1. Schistosoma mansoni in copula after D. R. Gönnert.

Ecology and Life Cycle (*Fig. 2*)

The normal habitat of mature S. mansoni is the hemorrhoidal and colorectal venous plexuses of man where the worm couples live in venules deep to the submucosa. Eggs are positioned as close as possible to the lumen, and exit is accomplished through histiolytic egg secretions, local necrosis, and peristaltic movements of the bowel. The precise mechanism of expulsion of ova remains unclear. The fate of eggs not reaching the lumen will be discussed below.

The egg that is passed in the stool contains the miracidium or embryonic form of the worm which on contact with water breaks out of its shell and seeks and homes onto the snail intermediate host, probably by chemotactic guidance toward the snail's mucus secretions. The snail hosts in America and Africa belong to the genus Biomphalaria and live in fresh to brackish water of lakes, rivers, sewers, and irrigation ditches.

Environmental control of the disease focuses on direct killing of the snail and on engineering to render unfavorable nutritive and other environmental conditions of its natural habitat. Such engineering is often at odds with agricultural and industrial needs. The Egyptians were dismayed to find that the bright promise of the Russian gift of the Aswan Dam was tempered with

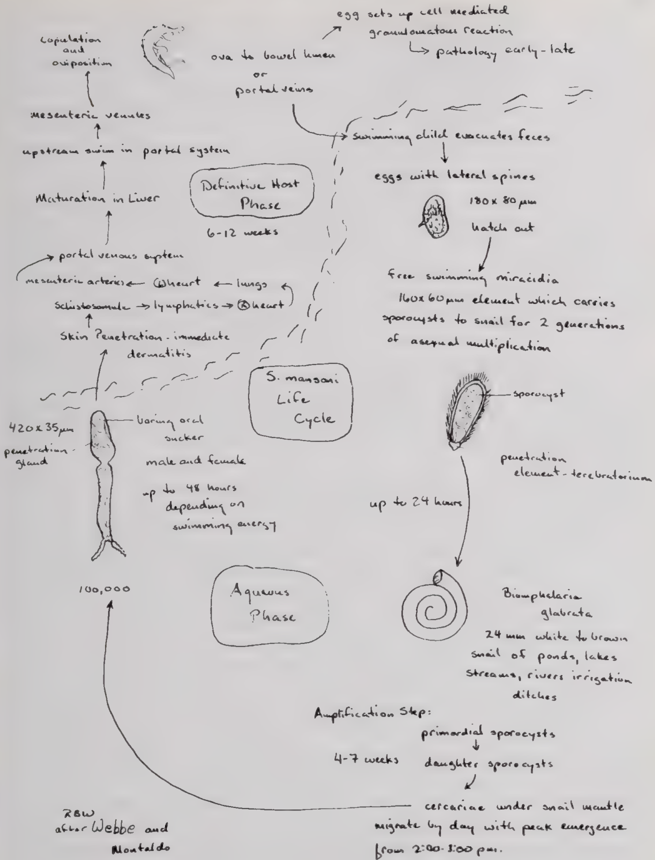
egg sets up cell mediated
granulomatous reaction
→ pathology early-late
copulation and oviposition
ova to bowel lumen or portal veins
mesenteric venules
upstream swim in portal system
swimming child evacuates feces
Definitive Host Phase
eggs with lateral spines
Maturation in Liver
180 x 80 µm
hatch out
6-12 weeks
portal venous system
mesenteric arteries ← Ⓛ heart ← lungs
Schistosomule → lymphatics → Ⓡ heart
free swimming miracidia
160 x 60 µm element which carries sporocysts to snail for 2 generations of asexual multiplication
Skin Penetration - immediate dermatitis
S. mansoni Life Cycle
sporocyst
420 x 35 µm
boring oral sucker
penetration gland
male and female
penetration element - terebratorium
up to 48 hours depending on swimming energy
up to 24 hours
100,000
Aqueous Phase
Biomphalaria glabrata
24 mm white to brown snail of ponds, lakes streams, rivers irrigation ditches
Amplification Step:
primordial sporocysts
4-7 weeks
daughter sporocysts
cercariae under snail mantle migrate by day with peak emergence from 2:00-3:00 pm.
RBW after Webbe and Montaldo

FIG. 2. Schistosoma mansoni life cycle after Webbe and Montaldo.

significantly increased rates of schistosomal infestation as miles of agricultural waterways were made accessible to parasite-carrying snails. When the free-swimming miracidium penetrates the snail target and changes form, becoming a sporocyst in 48 hours, its cargo of germ cells begins to multiply, and, after two weeks, primordial daughter sporocysts leave for the hepatic and gonadal tissues of the snail.

In these sites new germ cells multiply in the daughter sporocysts and develop into cercariae, free-swimming elements which then reinfect the definitive host. By the fourth week after initial snail penetration, the cercariae emerge with the onset of daylight and seek mammals swimming nearby. Amplification takes place since one miracidium may result in snail release of up to 100,000 cercariae.

Glycogen reserves are exhausted after 8 to 12 hours and the cercariae die if a suitable host has not been found. Cercariae penetrate the skin with lytic enzymes and active movements, and rest in the subdermis up to 4 days, undergoing change to the schistosomule or immature worm form. There is evidence that schistosomules take up host tissue antigens at this time and so are masked to immune surveillance. Migration most probably takes place via lymphatics and the superior vena caval inflow tract to the Ⓡ heart and then to the lungs, the major circulation, and finally the portal venous system to the liver. The migratory phase lasts 10 to 21 days. Maturation of male and female schistosomules takes place in the liver, after which the sexually mature worms swim to the distal venular feeders of the portal system and embrace during both copulation and oviposition, thus completing the life cycle at about 40 days after cercarial penetration. Adult worms can live up to 30 years in the human host.

Pathogenesis of Chronic Schistosomal Disease

In Puerto Rico the acute phase of illness is usually seen in young males who are exposed to heavily contaminated water. There may be an immediate dermatitis (swimmer's itch) caused by cercarial penetration after which there is a 16 to 60 day incubation period. Egg passage provokes the sudden onset of spiking fever with evening elevation, abdominal pain, and diarrhea, vomiting, weakness, myalgia, headache, anorexia, cough and weight loss.

The febrile period subsides spontaneously or with therapy by about the second week. The most effective treatment is with trivalent antimony compounds. The goal of treatment is often limited to reduction of worm load due to drug toxicity. Clinically there is generalized lymphadenopathy, soft tender hepatomegaly, and soft nontender splenomegaly. WBC is variably elevated, but there is always a marked eosinophilia. Hypergammaglobulinemia is present and abnormal liver function values are seen. Sigmoidoscopy reveals diffusely reddened edematous mucosa with pinpoint yellowish papules, small hemorrhages, and occasional small ulcerations. In cases coming to autopsy, small ulcerations may be seen as far proximally as the duodenum even though neither worms nor ova are found. Later duodenal and gall bladder lesions may mimic other UGI complaints. The above findings are probably caused by products of egg disintegration. Rectal biopsy during the ulcerative phase of the disease is frequently negative for ova, supporting this view.

The initial acute changes in the colon are confined to the lumen. With massive egg deposition over a long period, the pseudotubercular stage can progress to diffuse transmural fibrosis mimicing carcinoma, segmental colitis, or terminal ileitis. Massive intraluminal polypoid lesions are found more frequently in Egypt than in the Caribbean.

Murine models of infestation indicate how the chronic hepatosplenic phase of the disease begins. Up to 50% of the deposited ova may remain within the body, secreting the enzymes which facilitate their penetration into the gut lumen. Eggs may live up to three weeks, but provoke far less reaction than when they die. Eggs pass into the portal venous current along with breakdown products of RBCs consumed by the worms and worms that have died. Reaction to dead ova and worms takes the form of a periportal fibrosis secondary to resolving granulomatous lesions. As venous flow is progressively obstructed at the presinusoidal level, arterial neovascularation occurs and the resulting hepatic blood flow is little diminished. Accordingly hepatocellular damage is minimal and liver function is most often well preserved. However obstruction to portal flow causes the development of increased portal pressure with resulting hypersplenism, splenomegaly, and opening of portasystemic anastomoses, but no ascites except late in severe cases because sinusoidal pressure remains low.

The anastomotic channels predispose to later lesions. Esophageal varices are very frequent and massive bleeding from them may be a terminal event although schistosomiasis patients often seem to tolerate such bleeds much better than other cirrhotics perhaps because of their substantially greater hepatic reserve. Ova may be swept into the systemic circulation, bypassing hepatic filtration and causing extraportal granulomatous foci. Eggs trapped at the end arteriolar level of the pulmonary tree cause an allergic arteritis and granuloma formation with increase in pulmonary arterial pressure and eventually small vessel aneurysms that create AV fistulous connections with pulmonary venules as they resolve. These AV shunts lower arterial pO_2 and permit spread of ova beyond the lung filter with embolization to CNS, kidneys and other organs. In Egypt cor pulmonale is found in 2.1% of autopsied cases and pulmonary involvement per se in 30% according to El Mofty.

Brain granulomas are most often silent clinically as are those of the cord, but may produce vertigo, dizziness and/or epileptic seizures. Spinal cord involvement is the more frequent neurological manifestation and presents most often as transverse myelitis.

Renal lesions have received little attention although microhematuria is known to be present in schistosomiasis. Smithers states that worm somatic antigens may be found in patient urine. Andrade et al examined human autopsy material from cadavers with documented S. mansoni hepatosplenic disease showing focal to diffuse glomerular changes in 82% of cases characterized by mesangial cell proliferation, deposition of mesangial matrix material, and glomerulonephritis. Other renal changes found were patchy areas of chronic pyelonephritis, renal arteriolosclerosis related to cases with clinical hypertension, and cholemic nephrosis in cases with jaundice. The authors felt that the changes observed could be caused by deposition of antigen/antibody complexes at the basement membrane.

In summary the following picture of progressive schistosomal injury has been described: Stage I. hepatointestinal, beginning with acute diarrheal illness having the potential to progress to obstructive lesions of the colon and terminal ileum; Stage II. hepatosplenic, due to egg emboli of Stage I which produce portal vascular insult when filtered, leading to portal hypertension, splenomegaly, secondary hypersplenism, and finally opening of portasystemic anastomoses with probability of bleeding esophageal varices; and Stage III. ectopic granulomatous, with multiorgan involvement, CNS, and renal lesions being most often responsible for morbidity. It is important to note that all of these lesions may be tolerated for many years, allowing the parasite a stable, secure habitat in the definitive host to the benefit of the parasitic species. A case report demonstrating multisystem involvement at great personal and social cost will now be presented.

Case Report

When the patient was seven years old in Puerto Rico, he had his first known symptoms of a parasitosis at about the time that his mother died. He was known to have swum in the river near his home in a delta area. The initial symptoms were diarrhea, anorexia, epigastric pain, and headache. He was diagnosed and treated with an unknown agent with three injections per week for four weeks, but it seems without complete success for he was treated again in Puerto Rico at age fifteen. He was treated with penicillin for gonococcal infection at age 17. He arrived in the US at age 21 and was told that he had a bilharzia infestation, but he was well until five months prior to the first Beth Israel Hospital (NY) admission in 1949 when he began to have intermittent episodes of 2 to 3 days of epigastric pain after eating with occasional nausea and vomiting. Headaches and dizziness were frequent. There was no anorexia or diarrhea. Two weeks later symptoms became worse and there was cough without hemoptysis, fifteen-pound weight loss, fever and night sweats with occasional chills and chronic fatigue. There was no gross hematuria. Stool was negative on admission for occult blood but was positive for S. mansoni ova. Sigmoidoscopy revealed numerous small clusters of pink small pea-sized papules with overlying mucosa intact and no ulceration. Rectal biopsy disclosed many ova, several of which were seen to have lateral spines, and generalized granulomatous reaction. The patient was leukopenic with marked splenomegaly, lymphadenopathy, and eosinophilia-malaise with abnormal liver function tests until treatment with Fuadin was begun. A sternal marrow biopsy was seen to be normal with increased eosinophilic series. There were no signs of treatment toxicity, and after a three week hospitalization, the patient was discharged much improved for follow up in the medical clinic.

The patient became lost to follow up, but it is known that he broke his foot in Boston about 1950 and was successfully treated. He was admitted to Mount Sinai Hospital (NY) in 1954, and to Bellevue Hospital (NY) in 1961 for unknown reasons (the patient thinks that these admissions were for symptoms similar to those which prompted his first Beth Israel Hospital admission).

The second Beth Israel Hospital admission was in March 1963 for intermittent left upper quadrant pain of several months duration. Examination revealed petechiae distributed principally over the upper extremities, and a spleen palpable three fingerbreadths below the left costal margin. Marked finger clubbing was noted and there was a history of ankle edema. On laboratory examination, the patient was discovered to be leukopenic but not anemic. A bone marrow examination showed reticulum cell increase but was otherwise noncontributory due to insufficient specimen. It is interesting that the platelet count was depressed to 28,000 until prednisone therapy was initiated. At esophagoscopy a 1 cm bluish bulge suspicious for varix was seen on the anterior wall of the cardioesophageal junction. The patient had an episode of bizarre convulsive seizures which were thoroughly investigated without a definite conclusion as to organic or functional origin. The convulsive disorder was successfully managed with dilantin and phenobarbital.

There was a three-day period of passage of tarry stools without corresponding change in hemoglobin and hematocrit values which resolved upon administration of antacids. Serum electrophoresis showed hypergammaglobulinemia. CXR was normal. Bleeding time was three minutes, coagulation time six and one half minutes. Prothrombin time was 16.9/14.5, total bilirubin 1.63 and 0.99 direct, and BUN was 10. The patient was transferred to the surgical service and laparotomy was carried out. At the table a shunt was considered, however a splenectomy was performed because there were no intraabdominal signs of portasystemic anastomoses. Portal pressure by mesenteric vein cannulation was 40 cm H_2O before removal of a 780 gm spleen and 34 cm H_2O afterwards. Although there was blood with clots in the peritoneal cavity, especially the left upper quadrant on opening the abdomen, the pathologist's report contained no mention of a laceration of the spleen submitted for histology with a wedge liver biopsy. Microscopic findings were fibrocongestive changes in the spleen, and multiple granuloma without identifiable ova in the liver specimen. The patient was discharged improved, and was scheduled for follow up in the surgery clinic.

There was a brief admission for jaundice and possible wound infection 10 days after the second discharge. Serum hepatitis was diagnosed at outpatient visit about this time and it seems as if this resolved with outpatient treatment.

The patient was admitted a fourth time in November 1963 with complaint of hematuria of one month duration that was intermittent lasting one day every 3–4 days. There was occasional burning on urination but no history of colicky pain. ROS gave no history of weight loss or upper GI bleeding but elicited complaint of poor appetite and change of shoe size over a five-month period prior to admission. Physical exam was noncontributory. BP was 170/110. Lab values were: UA 3+ albumin with many RBC and WBC; urine culture results are not included in the microfilmed chart; H+H 12.7/38; WBC 6300 with 1 staff, 30 poly, 63 lymphs, 1 mono, 3 eos; ESR was 22; a positive latex fixation at a dilution of 1:340 was obtained; VDRL, Wasserman and PPD were negative; stool was negative for ova and parasites; there was low grade temperature elevation with pm spikes to 100° F as in past hospitalizations. Skull films and CXR were normal. IVP demonstrated a R double collecting system partially filled to the level of L_3. At cystoscopy a R ectopic orifice was seen in an essentially normal bladder. After injection of indigo carmine dye IV, good efflux was seen from all three ureteral orifices. Bilateral retrograde pyelography demonstrated good filling of the R collecting system, and unremarkable L collecting system. Multiple bladder biopsies showed nonspecific inflammation, and urine and bladder pap smears showed no malignancy.

During the hospital course, the patient was emotionally labile and had many crying spells and "hysterical reactions" characterized by jerking coordinated convulsions. These were managed with IM phenobarbital. A psychiatric consultation reported a lifelong history of nervousness and shakes, and a diagnosis of insufficient personality and hypochondria was made. An ophthalmologic consult for eye pain found normal eyes with early hypertensive retinopathy. The patient became afebrile but complained of pains all over his body which were said to have been well controlled with sterile water injections. The patient was discharged after nine days to be followed at the Mental Hygiene and GU clinics.

The fifth admission was in January 1964 when a neuroma was excised from the splenectomy scar and the diagnosis was confirmed on pathologic examination. A postoperative wound infection was treated with prostaphillin after culture returned coagulase-positive Staph. aureus. Laboratory values showed trace albumin in the urine and mild perturbation of liver function with normal total protein and albumin.

The patient was discharged on the thirteenth day and was thereafter seen many times in the ER for incisional pain and for an episode of chest pain all of which were treated symptomatically with donnatal and sterile water injections. Purulent wound drainage finally resolved. In January 1965 stool was negative for ova and parasites. In August 1966 a neurologic exam was normal. Stool for ova and parasites in May 1967 was found to contain numerous eggs of Trichuris trichiura, and treatment was instituted for a month with eventual return of negative stool for ova and parasites. Radiologic studies in July 1965 and January 1966 demonstrated normal barium enema but with poor preparation, and normal upper GI series with cascade stomach.

During the two years of outpatient visits, the most frequent complaints were poor appetite and weight loss eventually regained, episodic vomiting (one H+H value was 12.8/42-elevated for this patient), and left upper quadrant pain usually referred to the scar on physician notes with finding of a mass underlying the scar at times. Other complaints were frequent headache (a symptom of both S. mansoni and Trichuris infestation), constipation and flatulence. A clinic note entertained the diagnosis of recurrent neuroma and suggested ruling out peptic ulcer disease and pancreatitis. Relevant lab data over this period was as follows: WBC around 6500 with rough average differential of 50 polys, 35 lymphs, 4 mono, 4 eos; total bilirubin was less than 0.7. The patient admitted that there was a time in his life when he drank fairly heavily, perhaps during this period, but has always denied use of salicylates.

Outpatient sheets ended in 1967 and the documented history begins again with the sixth Beth Israel Hospital admission in March 1970 when a workup was done for hypertension. Admission VS were BP 190/100, P 75, R 20, and T 99F. Physical examination results differed only slightly from previous exams with the exception of mild gynecomastia, a just palpable liver, and mild pedal edema. Lab values were: WBC 5300 with 13 staff, 54 poly, 32 lymphs, 13 mono, 4 eos, 1 metamyelocyte and 2.6% reticulocytes; ESR was 12; UA pH 6. SG 1.025, 3+ albumin, 10–15 RBC, and 2–6 WBC; total protein was 5, albumin 2.4; PT within normal limits; ABG on room air pO_2 88, pCO_2 30, pH 7.4, 90% saturation; 24 hour VMA and catecholamines were normal; serum electrophoresis showed elevated beta globulinemia; LDH isoenzymes indicated RBC destruction or renal damage; VDRL was negative. CXR showed no active pulmonary disease and mild L ventricular hypertrophy. IVP demonstrated L before R renal parenchymal visualization, a double R collecting system and unremarkable L collecting system. Cystoscopy was negative without visualization of an ectopic ureteral orifice, both collecting systems were normal on retrograde pyelography. An ophthalmologic consult found early hypertensive retinopathy. Aldomet and hydrodiuril were begun with reduction of BP to 140/90. Creatinine clearance was 150 ml/min. The patient was suspected of having occult renal infection although culture of bladder urine obtained at cystoscopy was negative. The patient was discharged to Gouverneur medical clinic (NY) and GU clinic with a diagnosis of essential hypertension.

A seventh admission was in May 1970 to try to rule out a renal etiology of hypertension. Physical and laboratory findings were very similar to those on the previous admission with microscopic hematuria. Total bilirubin on SMA 12 was now 1.7, and H + H values were now higher at 13.6/40.4 VDRL and Wasserman were again negative and cystoscopy and L retrograde pyelography were essentially normal. Diagnosis at discharge was trigonitis and essential hypertension.

Personal history: mother died of TB when patient was seven, a younger brother died three years ago of what the patient thinks was a chronic illness similar to his own, family history otherwise noncontributory—a brother and sister are well in NYC; patient came to the US in 1947 and was often unemployed. He was divorced long ago and has a son fifteen by a common law wife, the son lives in Puerto Rico with a relative; the patient remained in the US until after his discharge from the hospital in 1970 when he returned home, however he made trips to visit relatives every 1–2 years during the time he was in the US.; he smokes 1–2 cigarettes per day and drinks two cans of beer a day; he wanted to become a hospital orderly but has been prevented from doing this by chronic ill health.

Present Beth Israel Hospital admission: A 49-year old Hispanic man was admitted at 2:00 AM to the ER with CC of two episodes of hematemesis (bright blood and clots) and orthostatic dizziness. The patient was in some distress. IV fluids were started, bloods were drawn, and the patient was admitted to the MICU.

HPI: The patient was moderately well in Puerto Rico until September 1974 when he had a similar episode of upper GI bleeding presenting with hematemesis. He was hospitalized at Aricaibo Hospital for four days of conservative treatment, and was discharged improved. The patient came to NY to visit his family two weeks prior to admission and had two episodes of UGI bleeding as above the night before he was to return to Puerto Rico.

In the MICU fluids were continued, an NG tube was passed, and iced saline lavage was begun returning dark blood, coffee ground material, and some fresh blood. Early in the morning of 8/22, panendoscopy was carried out with the finding of grade I varices with adherent clot just proximal to the cardioesophageal junction (CEJ). The mucosa at the CEJ was eroded. The stomach was found to contain blood in spite of lavage and the duodenum was found to be normal. It was felt that the source of bleeding was at the CEJ.

Admission VS were: BP 190/120, P 100, R 20, T 98° F.

Lab values were: WBC 8300 with 72 poly, 32 lymph, 5 mono; H+H 10.7 and 32.2; the smear showed blurr cells. ESR 10; SMA 12:diminution of total protein and albumin, calcium and phosphorous; augmentation of alkaline phosphatase (AKP) and LDH; glucose 140. BUN 33. UA; clear yellow, pH 6, SG 1.024, albumin 2+, many RBC, some WBC. PT was 14.6/11.3, PTT 30.3/31.8. ABG on room air was pO_2 72, pCO_2 32, pH 7.47, 94.7% saturation. Electrolytes HCO_3 27, Cl 107, Na 144, K 4.2.

ECG showed non specific QRS, ST segment, T wave changes.

CXR read essentially normal with some L heart enlargement.

The patient was transfused with 1 unit of A+ blood. A CVP line was inserted and the patient was transferred to the SICU for more intensive management.

On admission to the SICU a Sengstaken Blakemore tube was passed, the stomach balloon only was inflated and 200 gm of traction were applied. Soon after, continued lavage returned clear. By PM rounds on 8/22 VS were stable: BP 160/90, P 110, R 16, CVP 5-6, cm H_2O, Hct was 38, the WBC 20,000, glucose 195, BUN 30, electrolytes were within normal limits, urine output was minimal. The patient continued to be as alert and responsive as he had been since admission.

From the time of admission, the following medications were begun: valium IM prn, vitamin K, lasix IV prn to maintain urine output, aldomet, tylenol, MOM.

The patient soon stabilized on the above regimen. However on the second day after admission, he became jaundiced and had passage of melanotic stools. He was often withdrawn or disoriented possibly secondary to circulating bacterial metabolites of blood in the colon or the stressful environment of the ICU. The SB tube was unweighted then removed on the fourth day. Breath sounds were diminished at the R base, but other physical signs were fair except for a progressive rise in pulse and temperature.

By the fifth day, T was 102, ESR 60. CXR revealed extensive R lung infiltration with bilateral pleural effusion. WBC was 15,000 with marked left shift. BP 180/126, P 90, R somewhat labored, and CVP3 cm H_2O. UA dark amber, PH 6, SG 1.015, albumin 1+, occult blood +. Bilirubin was 7.1 total and 2.3 direct, total protein 4.3, albumin 2.7. AKP, CPK, GOT elevated, LDH 600. PT and PTT were near control values. Glucose was 120, BUN 14, electrolytes: HCO_3 25, Cl 106, Na 134, K 4.2.

Bloods for culture drawn earlier grew C. perfringens and E. coli, the sputum Staph. aureus. Stool was negative for ova and parasites. A CVP tip had grown nothing at 48 hours. IV keflin was begun. Temperature peaked at 104 on the seventh day accompanied by a WBC of 19,000. A hypothermia blanket was applied and the temp was brought to 101^4 by pm rounds. Urine output had dropped and the ABG values had been: pO_2 65, pCO_2 37, pH 7.49, saturation 73.5%. A culture of blood had grown coagulase + Staph. aureus. UA was cloudy, SG 1.013 and positive for albumin and occult blood. NH_3 was 59 and other liver function values were abnormal as above, but coagulation times remained good. Blood continued to be transfused as indicated. Electrolytes were acceptable. Enzymes remained markedly elevated. H+H were adequate with occasional target cells and schistiocytes seen on the smear.

By the twelfth hospital day, temperature was below 100F. The WBC was 8300 with 13 staff, 71 poly, 14 lymph. Serial CXR showed resolving R lung infiltrate andⓇpulmonary hypervascularization. H + H values declined but were brought to 10.6/32.2 following transfusion. Physical findings were acceptable but disclosed 2+ leg edema. Total protein and albumin remained low and albumin was given IV. BUN was 13, glucose 100, and electrolytes were indicative of mild hemodilution. Total bilirubin and enzymes remained moderately elevated. The patient was deemed ready for transfer to Dazian 7W and was advanced from sippy to a soft bland diet.

The patient continued to improve clinically and on laboratory and serial CXR. Stool was negative for occult blood, ova, and parasites, and blood and urine cultures returned without growth. A sputum culture grew H. influenzae. CBC and 12-channel hovered at adequate levels, but mild reticulocytosis was seen on the smear. ESR remained elevated and temperature varied between 99 and 100 F. H+H stayed at 11-12/34. Enzymes were mildly elevated. Total protein and albumin fluctuated near normal. UA was clear yellow, pH 5. SC 1.015, there was trace albumin and some RBC and WBC. CXR was read as normal.

An infectious disease consult suggested beginning oxacillin, and there was continued improvement with this treatment; bringing temperature to under 99° F. AKP remained mildly elevated, but other parameters of liver function were satisfactory and clinical jaundice had disappeared. IV fluids were discontinued, and the soft diet was advanced to low protein low salt. The patient complained of headaches and constipation, but his mental status returned to pre-illness levels. A portasystemic shunt procedure was proposed to him and a psychiatric consult felt the patient was reacting with appropriate anxiety to the anticipated surgery although the affect was depressed/melancholic.

On the fifty-fourth day in hospital, selective superior mesenteric arteriography was performed with caval and hepatic venous mannometry. The aortic flush revealed a distorted abdominal arterial tree and thinning of the Ⓡ renal small arteries. The splenic artery was absent. The venous phase of selective superior mesenteric artery visualization showed no inferior mesenteric vein reflux and good filling via the portal vein of a very shrunken liver. Esophageal varices were faintly seen. This venous flow pattern was interpreted as consistent with fair emptying of the portal venous system. Inferior vena cava pressure was 6 cm H_2O, Ⓡ atrial pressure was 5 cm H_2O, and WHVP was 5 cm H_2O indicating presinusoidal obstruction. There were technical difficulties and poor patient tolerance (emotional) of the long procedure which precluded more complete studies, but the radiologist felt that the results oriented away from the necessity of a shunt for variceal decompression.

Student physical exam on the fifty-third hospital day revealed a thin, chronically ill-appearing man in no acute distress. He was somewhat anxious about being reexamined and so the physical was carried out over several days in the course of talking with the patient so as not to upset him unduly. Wt 130 lb, Ht 5'8".

HEENT: normocephalic without cranial nodes, thick graying hair extremely well groomed. EOM's full, PERL, Fundi not very well seen, sharp disc margins bilaterally, frequent arterial silver wire aspect with some AV nicking. No hemorrhages or exudates seen. Presbyopic, corrected with glasses. No scleral jaundice, conjunctiva pale. TMs not done, nose and throat clear, no sublingual jaundice, poor teeth, fecal breath, pale lips, tongue fibrillations without atrophy.

Neck: a few shotty nodes appreciated bilaterally, no supine venous congestion seen, carotid pulses 2+ bilaterally without bruit.

Chest: no asymmetry or deformity, possible very mild gynecomastia, lungs clear to P+A, some shotty axillary nodes on R.

Cor: no lifts or heaves seen, PMI just below Ⓛ nipple, $S_1 << S_2$ NSR, no other sounds or murmurs heard.

Abdomen: thin and flat with a long Ⓛ subcostal scar from the midline to the flank, well healed. No venous markings seen. Soft NT. A firm linear mass was felt oriented vertically from the L iliac fossa to the L hypochondrium which was about two fingerbreadths in width and was not tender. The liver was not palpable but was percussed to a fingerbreadth above the costal margin. No other masses, no hernias. Rectal exam revealed external hemorrhoids, no stricture or stool, the prostate was not appreciated.

GU: normal male without testicular atrophy.

Extremities: very thin with 4+ finger and toe clubbing, no palmar erythema, there were some shotty inguinal nodes bilaterally. Radial and dorsalis pedis pulses were 2+ bilaterally.

Neuro and mental status: noncontributory after extensive testing, specifically no asterixis, ataxia, or change in handwriting from old chart consent forms. Patient well oriented to time and place and many details of his youth and past hospitalization. Communication was somewhat hindered in spite of good patient command of English. Patient was initially very anxious but was calmer after discussion of his medical status although he became overwrought during angiography and was reduced to tears.

VS were BP 160/110, P 80, R 18, T 99^4 and ROS over the course of the examination was noncontributory except for occasional mild headache and chronic constipation. Medications during the covalescence were hydrodiuril and apresoline, SSKI, $FeSO_4$, castor oil, compazine, benadryl, and tylenol.

Summary: the patient has been shown to have a long history of treatment of sequelae of childhood and early adult infestation with S. mansoni. It is the student's impression that in addition to documented portal hypertension and treated hypersplenism and splenomegaly, he has had a chronic renal problem which is only marginally compensated. This belief is reinforced by long-standing findings of gross and microscopic hematuria and variable albuminuria. The question of whether this patient's past neurologic and emotional difficulties are secondary to the sequelae of past granulomatous cerebral disease or are due to the life circumstances of a chronically ill person is difficult to answer.

Surgical Treatment of Schistosomiasis

Historical landmarks in the surgery of portal hypertension are discussed by Warren and begin with the Russian military surgeon Nicoli Eck who demonstrated technical feasibility and animal survival of a shunt in 1877. McIndoe laid the foundations of the vascular physiology of cirrhosis about 1928. In the late 1930's and early 1940's, Allen D. Whipple at Columbia documented human elevation of portal pressure and demonstrated good liver function in patient's with extrahepatic thrombosis leading to the belief that diversion of portal venous flow could be tolerated in man. At about the same time, George Whipple at Rochester elucidated the metabolic aspects of shunt in dogs used for the study of protein metabolism and anemia. He communicated his findings to Dr. Whipple at Columbia who, thus encouraged, undertook the first splenorenal shunts in the early 1940's.

Because schistomiasis patients generally have good liver function, they are good candidates for the procedure. They rarely manifest encephalopathy even after multiple episodes of bleeding (one patient tolerated 34 such episodes). The probable reason for this is that they have good arterial compensation for portal obstruction and are able to effectively clear NH_3 and toxic metabolites of amino acid breakdown by colon bacteria in a way not possible for other cirrhotics. In fact, on the basis of functional hepatocellular mass, they ought not to be classed with cirrhotics at all according to Aufses et al. Raffucci et al in their series claim to have much better results after shunting procedures in these patients and De Lima et al have shown that total hepatic blood flow returns to already adequate preoperative levels after shunt or splenectomy.

In the past patient's were prepared for shunt with a repeat course of antimonial chemotherapy so that creation of the new high volume portasystemic connection would not flood the systemic circulation with ova and worms. Goldsmith has devised an elegant alternative which eliminates the need to embolize more parasites to the liver preop. At operation, which is performed initially under epidural anesthesia to preclude premature worm migration, an extracorporeal double-filter system is connected between the portal vein and a cutdown to a peripheral vein. IV antimony potassium tartrate is administered, and worms are induced to migrate from the mesenteric venules to be trapped by the filter. As many as 150 parasites may be recovered in this way over a 60-minute period with small additional risk to the patient. This method probably does not remove all the worms present and some may slip by the filter, but reported results have been good. Recently a Mount Sinai patient whose case was presented at grand rounds underwent the procedure at elective surgery for varices and tolerated the procedure well.

Of all the types of procedures, many feel that the distal splenorenal shunt procedure of Warren is best in terms of decreased postoperative encephalopathy and preservation of liver function, but the coronacaval shunt of Linton, while more technically exacting, seems to have the soundest foundation in theory and has given very good early results.

The shunt being envisaged for the patient presented above is a mesocaval H type with dacron graft. Because this procedure is fast and technically simple, it is indicated when an emergency shunt must be carried out for uncontrollable bleeding.

If materials technology can solve the problems of creating nonthrombogenic tubing and stable transcutaneous plug appliances, an attractive method in terms of technical simplicity (emergency procedures) and the possibility of patient connection to dialysis or immunoadsorbants of toxic materials would be a portasystemic bypass graft. Such a system would be especially valuable in cases of late hepatic decompensation, a common terminal event in shunt patients. Important postoperative complications of shunts are thrombosis and encephalopathy (shunt patient's are managed on low protein diets and intraluminal antibiotics may be used) and decreased liver function due to diversion of hepatic blood flow.

Some authors prefer a direct attack on the varices by ligation, partial gastrectomy, or injection of sclerosing solutions especially in emergency situations. As with shunts, emergency use of these methods carries high mortality. Shunt procedures are more effective for definitive control of varices although recurrence of bleeding may occur with them as well. Mortality for elective shunt is about 15% depending on the series.

Other surgical procedures in schistosomiasis are splenectomy for the indications described above and to partially decompress varices by decreasing arterial input to the portal system; segmental resection of the colon for obstruction due to scarring or very large pseudotubercule; and colectomy in cases where encephalopathy is a severe problem with or without a shunt. Liver, rectal and bladder biopsies are an important means of establishing the diagnosis although they are likely to be replaced with immunologic methods such as quantitation of host lymphocyte proliferation upon confrontation in culture with cercarial antigen. Ideally, experimental control of the parasite and nontoxic effective chemotherapy would remove this disease from the realm of the surgeon, but these goals are still far off and management of the increasing incidence of S. mansoni infestation will probably remain of surgical interest for many years to come.

Advisability of a Shunt in the Above Patient

There are unanswered questions about the exact nature of the patient's disease. It would be interesting to see results of R heart catheterization in this patient to determine whether pulmonary vascular lesions could be responsible for his low pO_2 values. Renal biopsy would be desirable to document whether his renal problems and hypertension are related to glomerular deposition of antigen/antibody complexes.

It would be worthwhile to do BSP retention and hepatic vein O_2 saturation studies to get a better picture of hepatic reserve. The problem of whether esophageal varices are definitely the cause of bleeding in the patient remains. The findings at esophagoscopy with the presence of clots and blood obscuring vision seem equivocal. Sandblom presents strong evidence that portal hypertension can cause bleeding in up to 10 % of cases by a hemorrhagic gastritis or bleeding ulcer. Congested stomach mucosa seen in these patient's is envisaged to be far more susceptable to injury from alcohol, salicylates or the effects of stress related gastric hypersecretion. Sandblom goes on to say that there is evidence that varices bleed not only from increased intraluminal pressure but also from erosion of overlying mucosa. Certainly this patient has had indications of gastritis in the past such as postprandial epigastric pain, and episodes of tarry stools during a stressful time which disappeared with antacid therapy. Does the isolated finding of a normal serum gastrin rule out this alternative explanation of upper GI bleeding? Finally if this patient is operated it would be interesting to use the Goldsmith procedure to see if, in spite of repeated negative stools for ova and parasites (perhaps due to extensive fibrosis preventing egg exit), worms are still living in his body.

Should this patient be operated at this time? If his bleeding source is not varices, (in 1963 he had only suspicion of one in the face of an already elevated portal pressure) then medical management to allow mucosal healing and to blunt the effects of stress would be indicated. If in fact the bleeding source is varices, will the patient be able to derive long-term benefit from a shunt? His fair nutritional status, lack of ascites or encephalopathy, and relatively low bilirubin with mildly depressed albumin place him between the good to moderate risk categories of Turcott. He is not an alcoholic, but this same author has shown evidence that there is no significant difference in the somewhat discouraging three-year survival figures between alcoholics and other shunt patients. It is not accurately known how good the portal blood flow is to this patient's shrunken liver, and the evidence for loss of hepatocellular mass postshunt due to decrease in delivery of a postulated anti-atrophic factor must be taken into account.

This patient's previous reactions to hospitalization and his desires to return as quickly as possible to Puerto Rico are also important factors in a decision about surgery. All of these considerations lead me to believe that the best course for this patient might be return home for further studies. At their completion, a final decision could be made as to whether or not a shunt procedure to be done in Puerto Rico is indicated.

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Student Section

Indications for Surgical Intervention in Children with Ulcerative Colitis

ELLEN WOLFSON, B.A.

Approximately 10 to 15% of patients with ulcerative colitis (UC) have onset of the disease in childhood (1, 2). There are many reasons why the pediatric patient with UC should be considered separately from his adult counterpart. Children with UC tend to have more severe disease than adults. Binder et al (1) found 90% of their pediatric patients had moderate or severe disease compared to only 50% of adults. Similar findings were reported by Watts and coworkers (3) and by Patterson (4). Children with colitis almost always have a total colitis, rarely the more benign proctocolitis found in one-third of adults with UC (5). Children frequently have a higher incidence of extracolonic manifestations of the disease (anemia, arthritis, uveitis, skin lesions, etc.) than adults—37% vs. 25% in one series (1). Children with UC have more of the identified risk factors for developing carcinoma: total involvement of the colon, more severe first attack, longer duration of disease (6). Finally, children with UC may suffer from a profound disturbance of growth, development, and sexual maturation.

Proctocolectomy cures ulcerative colitis but at the price of a major operation and life with an ileostomy. The most important decision the physician faces in treating patients with UC is when to abandon medical therapy and to advise radical surgery. The proposed indications for surgery in children with ulcerative colitis can be summarized as follows:

I. *Acute attack:* life-threatening situations such as perforation, hemorrhage, toxic dilatation, fulminating disease not responding to medical therapy.
II. *Elective Surgery*
    A. *chronic disease:* "chronic invalidism," severe retardation of growth and/or sexual maturation; certain complications such as suspicion of carcinoma, severe fistula, intractable arthritis, uveitis, skin lesions, etc.
    B. *prophylaxis:* elective colectomy to eliminate risk of carcinoma or to improve long-term prognosis.

Emergency Surgery for Ulcerative Colitis

Undisputed indications for emergency surgical intervention include uncontrollable hemorrhage, perforation with peritonitis, toxic megacolon, and

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fulminant life-threatening disease not responding to medical treatment. The importance of early surgery in these patients is stressed by the findings of Goligher and coworkers (7) who have lowered the overall mortality of severe attacks of UC from 11.3% to 4.5% and the operative mortality from 20% to 7% by adopting a policy of performing definitive surgery in all patients not responding to medical therapy within 5 days.

Toxic colitis, or "megacolon" is potentially the most lethal of all complications of UC, and its early diagnosis and treatment are crucial if the patient's life is to be saved. A trial of intensive nonoperative treatment is indicated with the patient watched very closely for actual or impending perforation. Surgery is indicated for perforation, deterioration of clinical status, or failure to respond quickly to medical therapy. There is some controversy over the type of surgery to be performed. When operating on a severely debilitated patient with impending perforation, many authors recommend the more conservative subtotal colectomy with ileostomy, and creation of a rectosigmoid mucous fistula which can be removed later under elective conditions (7).

Severe hemorrhage in children is uncommon and surgery is rarely required for this complication. If hemorrhage is uncontrollable, the procedure of choice is total proctocolectomy with abdomino-perineal resection, since a lesser procedure with preservation of the rectal stump risks continuing hemorrhage from the remaining colon. (7)

Relief of Extracolonic Manifestations of UC

The principal extracolonic complications of UC affect the joints, eyes, and skin. About 7–10% of patients develop a peripheral arthritis, while another 3–4% have ankylosing spondylitis and/or sacroileitis. The principal ocular complications are episcleritis and uveitis; clinically significant cases occur in 3–4% of patients with UC. Approximately 5% of patients develop skin lesions, the common types being erythema nodosum and pyoderma gangrenosum. In addition to the classic colitis lesions of joints, eyes, and skin, other systemic complications include liver/biliary tract disease (chronic hepatitis, cirrhosis, sclerosing cholangitis, bile duct carcinoma), kidney stones (25 times greater incidence than the general population), and anemia (8).

Sacchar and Janowitz (8) report that some of these lesions are not relieved by surgery. They find that ankylosing spondylitis as well as some hepatic lesions may persist unabated after total colectomy. Furthermore, the occurrence of renal stones may even increase somewhat after surgery.

However, Ehrenpreis et al studied the incidence of extracolonic lesions in 23 children before and after surgery and noted the following results (9):

	preoperative	*postoperative*
arthritis, arthralgia	7	1
erythema nodosum	10	0
stomatitis	4	0
anemia (Hgb <9 gm %)	22	0
liver damage	3	0

On the whole, the data suggest that intractable suffering from most of the extracolonic manifestations of UC can be completely relieved by surgery.

Growth Retardation

Growth retardation has been reported in children suffering from many types of chronic debilitating diseases, including ulcerative colitis. Berger et al (10) studied 85 children with UC, 37 of whom had sufficient height data to evaluate the influence of the disease on growth. Of these 37 children, 21 (57%) were in the 10th percentile or below in height, with 11 of these (29%) in the 3rd percentile or below. Similarly, McCaffrey and coworkers (11) studied 130 children with inflammatory bowel disease and found 22 (17%) to be below the 3rd percentile in height.

Lyons and Baronofsky (12) studied the effect of surgery on five children with UC whose growth and sexual development were severely retarded. One patient who was operated on at age 10 (2 years after onset of disease) has since attained relatively normal height and weight, and has achieved puberty. The four other patients, who underwent surgery between the ages of 15–17, have all failed to achieve normal growth or sexual development. The authors interpret these results to indicate that a child with retarded growth who enters the pubertal years with diseased colon intact has a very poor chance of recovering growth or sexual maturity—even if the diseased colon is removed later on.

McCaffery et al (11) operated on 11 children with inflammatory bowel disease (UC and GC) who were below the 3rd percentile in height. Three of 11 children achieved an increase in percentile height following surgery. In contrast to the findings of Lyons and Baronofsky, no significant difference was found between children undergoing surgery before versus after puberty (1 of 3 children operated on before age 12 grew after surgery compared to 2 of 8 children operated on after age 16).

Voinchet and coworkers (13) compared medical versus surgical treatment for 16 growth-retarded children with inflammatory bowel disease (10 with GC and 6 with UC). All children were originally below the third percentile in height. Of 6 patients treated medically, 2 have achieved normal height upon reaching adulthood. Only 4 of 10 patients treated surgically showed a greater than 100% yearly growth rate, and 6 of 7 who are now adults remain in the 3rd percentile. Thus, in this study, no significant difference was observed between medical and surgical groups (nor between UC and GC). The authors conclude that growth retardation as the sole or major indication for resective surgery in patients with UC does not seem justified.

Berger, Gribetz, and Korelitz analyzed growth patterns in 37 children with UC, and compared the effects of medical vs. surgical treatment. They found a significant difference between the "medical" group where $^{3}/_{15}$ patients (20%) achieved a noticeable increase in percentile height, and the "surgical" group where $^{8}/_{16}$ (50%) had a percentile increase in height. Furthermore 6 of the 8 surgically treated patients who failed to demonstrate a percentile height increase did show an increase in growth velocity after surgery, which may influence the adult height they eventually attain.

If Berger's data are analyzed separately for children with severe growth retardation (≤ 3rd percentile) the results are even more striking. Of 4 children in this category who were treated medically, none showed a percentile height increase, whereas 3 of 7 children treated surgically achieved a noticeable increase in percentile height.

Furthermore, Berger's study supports the conclusion of Lyon and Baronofsky that, to be effective in relieving growth retardation, surgery should be performed as early as possible. 8 of 10 children (80%) operated on before age 13 showed a percentile height increase compared to only 1 of 4 children (25%) whose surgery took place at age 13 or older.

My recommendations based on the conflicting data in these four studies are as follows:

1. severe growth retardation (≤ 3rd percentile) in a prepubescent child is a valid indication for colectomy.
2. mild or moderate growth retardation in a prepubescent child is a questionable indication for colectomy.
3. growth retardation in a child whose epiphyses have closed or who has passed the chronologic age of puberty is not an indication for surgery.

Chronic Invalidism

Chronic invalidism and debilitation has been accepted by many authors as an indication for elective surgical intervention. Ehrenpreis and coworkers (9) state that surgical intervention should be considered in children and young adults who, despite medical treatment, have been totally disabled by their illness for more than one year, or partially disabled for more than 5 years. The widely held opinion that an ileostomy would constitute a severe handicap for children and adolescents is not borne out by the experience of many authors (5, 9, 14, 15, 16). For example, Ehrenpreis (9) found the 23 children in his study all preferred their present condition to that before surgery and did not feel the ileostomy to be any serious drawback.

Prophylaxis against Carcinoma

It has been suggested that prophylactic colectomy should be performed after 10 years of UC in order to prevent the alarming incidence of carcinoma in this high-risk group. Devroede and coworkers (6) followed 396 patients suffering from UC from childhood for periods as long as 43 years, and studied their survival and risk of developing carcinoma of the large bowel. Among their patients who survived without proctocolectomy, carcinoma developed in 3% by 10 years, 23% by 20 years, and in 43% by 35 years after onset of UC.

Edwards and Truelove (17) identified certain factors which they found were identified with an exceptionally high risk of carcinoma. These include: clinically severe first attack; radiologic evidence of universal involvement; chronic continuous symptoms; and onset in childhood or early adult life. These authors suggest that a patient who shows a combination of 2 or 3 of these high-risk factors should be considered for a colectomy even if there is no other indication (18).

Not only is the incidence of colorectal carcinoma increased in UC, but according to Sacchars and Janowitz (8) it carries a worse prognosis that similar cancer occurring in the general noncolitis population. In patients with UC, carcinomas occur at a much younger age (average 38 years versus 58 years in the general population), more often are multiple (23% vs. 5%), and are more often aggressive, metastatic and fatal.

It has been suggested that regular followup with sigmoidoscopy and barium enema, with immediate surgery for all suspicious lesions, is sufficient to deal with this problem and obviates the need for prophylactic panproctocolectomy (D. Gribetz, personal communication). However, several authors point out the difficulty of detecting carcinoma in patients with severe and extensive bowel disease (8, 9). For this reason, efforts have been made to develop more sensitive screening tests for colonic carcinoma in UC. These include cytologic examination of colonic washings, and study of rectal biopsies for certain "precancerous" changes. Neither method has as yet achieved widespread clinical application. Assays of blood samples for elevated levels of carcinoembryonic antigen (CEA) may be useful when used to follow patients over long periods of time. However, they are not useful as one-stage screening tests since CEA levels are elevated in about 40% of patients with UC, and in more than 85% of patients with severe colitis, irrespective of known cancer risk (8).

Thus the issue of proctocolectomy as prophylaxis against carcinoma remains a controversial one. Since the incidence of carcinoma in the first 10 years is only 3%, I feel that prevention of carcinoma as the sole indication for surgery within the first ten years is not justified. Such patients should, of course, be scrupulously followed with sigmoidoscopy and barium enema at least every six months. In this way, the 97% of patients who will not develop cancer within the first 10 years are spared at least a few years of life without a colostomy. It is only after the first ten years that the risk of morbidity and mortality from carcinoma exceeds the morbidity and mortality of elective colectomy.

Surgery To Improve Long-Term Prognosis

Two studies have analyzed the long-term survival rates of medically versus surgically treated children with ulcerative colitis. The results are shown below:

		Devroede (6)		*Bonnevie (19)*	
		med	*surg*	*med*	*surg*
% Surviving	5 yr	90	82	97	83
	10 yr	78	78	94	83
	20 yr	47	78		

These data indicate that both 5- and 10-year survival rates are slightly higher for medically treated patients. This finding seems reasonable when one considers that patients who require surgery are generally sicker than those who can be treated medically. Furthermore, much of the surgery performed early in the course of the disease is for emergency indications (with a 15–20% mortality rate) as opposed to elective surgery, which carries only a 3–5% mortality rate (2).

However, the data for 20-year survival shows a striking difference between the two groups—78% survival for surgically treated patients vs. 47% survival for medically treated patients. This figure probably reflects the higher incidence of elective rather than emergency surgery in the later stages of the disease, as well as the high risk of carcinoma once the 10-year mark is passed.

It would require a large-scale, randomized, long-term study to fully evaluate the role of surgery in improving prognosis of UC. However, on the basis of existing data, surgery to increase long-term survival rates should be recommended, if at all, only to patients 10 years or more after onset of the disease.

Conclusion

In the absence of data from long-term randomized large-scale studies, few statistically valid conclusions can be drawn about indications for surgical intervention in children with ulcerative colitis. On the basis of existing data, however, certain recommendations can be proposed. The child with UC should be handled by a team approach. At the time of initial diagnosis of the disease, the child and his family should meet with a group consisting of a pediatrician, surgeon, radiologist, psychiatrist and social worker to discuss and decide upon a plan of therapy. The child's progress should be closely monitored. Periodically the entire group should meet to assess the child's total condition, both physical and emotional. Factors such as ability to control symptoms with medical therapy, growth and development, presence of extracolonic manifestations, as well as emotional well-being, social adjustment, and functioning in school should all be considered. Regular physical examination with barium enema and sigmoidoscopy should be performed to aid in early detection of cancer.

The key to managing these children is to allow time for an adequate evaluation of response to medical therapy, but not to postpone surgical intervention until problems have progressed too far to be corrected. If surgery is elected for severe growth retardation, it should be performed before puberty. The child with severe arthritis, skin lesions, or liver disease should have surgery before irreversible damage is done, and the child with intractable debilitating disease should have surgery before he becomes a psychosocial invalid. On the other hand, surgery to prevent risk of colorectal carcinoma or to improve long-term survival is not indicated until at least ten years after onset of the disease.

Colectomy and ileostomy in many children with ulcerative colitis should not be regarded as a dreadful last resort, but as relief from a chronic disabling disease and its many complications.

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Letters to the Editor*

ON ANAL FISSURES

Dear Sir:

Doctor Changyul Oh's paper on anal fissure (Mount Sinai J Med 42:596, Nov-Dec 1975) necessitates a response for a variety of reasons. It brings to mind Alexander Pope's "Be not the first by whom the new is tried nor yet the last to lay the old aside," and the well-known axiom "The expert is seldom in doubt but frequently in error." The main error lies in the failure to precisely recognize and point out that anal fissures declare themselves in three forms, namely: 1) the very superficial fissure that in reality is a mere abrasion; 2) the deeper but still superficial fissure with negligible or no involvement of the underlying tissues, and 3) the deep fissure that is associated with subjacent and adjacent induration, a sentinel pile (apparently only ten such instances in Oh's series), an anal fibroepithelial polyp with an inflamed or infected anal crypt, and decided involvement of the anal sphincteric musculature. The pathologist describes this lesion as a chronic ulcer. The associated pronounced anal spasm is caused by irritation and involvement (including fibrosis) of at least the lower border of the internal anal sphincter muscle as well as the frequently ignored or overlooked muscularis submucosae ani described by Fine.

Recognition of the foregoing leads to effective and rational treatment. Neither type of the superficial fissures requires surgical intervention, whereas the chronic anal fissure invariably calls for a surgical procedure which incudes an adequate *open sphincterotomy. I had demonstrated and profusely illustrated this type of operation in 1948 (Surg Gynec Obst 86:434, April 1948). I have performed this operation on hundreds of patients. Following complete healing of the wound, though I am aware of the possibility, I have not encountered "minor complications such as stool soiling" unless the sphincterotomy is performed anteriorly in women who have borne many children because the important levator ani muscle has practically no representation anteriorly.*

An occasional recurrence following the open operation is probably the result of faulty technique. Oh has stated that he has not encountered recurrence (p. 600) in his 100 cases. This is not so in his patient (ZR) on whom he performed a second operation on November 28, 1975 at Mount Sinai Hospital upon my advice. This is not a solitary experience.

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* Letters should be clearly marked "For Publication" and will be published at the discretion of the Editor and as space permits. Letters are subject to editing. Letters for publication should include name, degree, chief institutional affiliation, and address of author. Mail Letters to: Editor, *The Mount Sinai Journal of Medicine*, The Annenberg Building, Room 10-35, Fifth Avenue and 100 Street, New York, N. Y. 10029.

One may take issue with Oh on the wisdom of favoring closed anorectal wounds in civilian practice. Note, though, that eventually he modified or abandoned this practice as judged by his advocacy of "iodoform gauze (packing) in order to eliminate hematoma and infection, and other complications." During World War II, I had an opportunity to study and compare the open, semiclosed and closed wounds following uncomplicated hemorrhoidectomies performed in a military hospital. The high incidence of wound complications warranted the discontinuance of the closed technique even though it was important to return soldiers to active duty as early as clinically possible (S Clin North America 32:677, April 1952; Treatment in Procotology, *Williams and Wilkins Company, Baltimore 1949). The open type of operation, though it entailed a longer healing time, was the preferred technique. Parenthetically, the addition of chemotherapeutic agents was of no help in the prevention of complications following the closed technique.*

As a rule, one does not take issue with a qualified surgeon who employs routinely a procedure, no matter how unorthodox it appears to others, which in his hands is safe and productive of superior end results. However, it is quite a different matter when such a technique is published because, by common understanding, publication is tantamount to an advocacy or recommendation which at times may be a trap for the unwary or inexperienced surgeon.

I have not kept statistics on this subject, but my long and rich experience leads me to the conviction that a lateral sphincterotomy is an unnecessary gimmick for the treatment of either type of superficial fissure because these anal lesions respond promptly and well to nonsurgical measures; this operation, on the other hand, is hardly ever effective for the deep, chronic anal fissure (ulcer). The cure of this lesion calls for an open type of operation similar to the one I illustrated as far back as 1948. Alexander Pope was right!

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Dear Sir:

In response to Dr. Turell's comments on my paper entitled "Lateral Subcutaneous Internal Sphincterotomy for Anal Fissure" (Mount Sinai J Med 42:596, 1975), I wish to present the following analysis.

This technique, subcutaneous sphincterotomy, had already been published in a number of journals since 1967 (1) and had been practiced even earlier by Miles in 1939 (2). Dr. Turell comments as though I were the first to introduce the technique. I merely reintroduced it with minor modifications.

I do agree with Dr. Turell that acute or superficial ulcers should be treated with conservative methods, while chronic intractable ulcers would benefit *from the surgical approach. However, he goes on to say that only 10 cases of my series are candidates for surgery; and yet I clearly described the material and concluded by stating "100 cases of chronic, intractable anal fissure."*

I am very surprised to learn that Dr. Turell has not encountered a single case of minor fecal soiling during his "long and rich experience" in the treatment of anal ulcer. Contrary to this, outstanding surgeons have reported a 30% incidence of fecal soiling following open posterior sphincterotomy (3–5).

Dr. Turell points out that one of my cases (ZR) developed recurrence following surgery. This case was not included in the reported series; the paper was submitted in September 1974 whereas the operation took place on March 18, 1975 and there was recurrence in November 1975. Recurrences will be seen more often as our experience and length of follow-up increase.

Closed method versus open method is still controversial. The Ferguson Clinic has adopted radical hemorrhoidectomy with primary closure since 1930 (6). Among the 2,038 cases, the immediate postoperative abscess formation was 4 cases, and a five-year follow-up revealed perianal abscess in 13 cases and fistula in ano in 10 cases. No one can argue against successful operations. While I agree that the open method is safe and secure, I do not think it is fair to condemn the closed method without personal experience. It is well documented that the spasmodic contraction of anal ulcer can be successfully treated

TABLE I

Incidence of Minor Disturbances of Anal Continence after Various Operations for Anal Fissure

Type of Operation	No. of Patients Reviewed	Percentage of Incidence of Minor Defects of Anal Control				
		Impaired Control for		Fecal Soiling of Underwear	One or More Defects	Recurrences
		Flatus	Feces			
Open, posterior internal sphincterotomy (Bennett and Goligher, 1962)	127	24	11	28	45	7
Lateral subcutaneous internal sphincterotomy (Hoffman and Goligher, 1970)	99	6	1	7	12	3

TABLE II

Results of Subcutaneous Internal Sphincterotomy

Reference	No. of Pts.	Hosp. Stay	Healing Time (Wks)	Cure Rate (1%)	Minor Defect (1%)
Hawley ('69)[14]	24	5	3	100	0
Notaras ('69)[8]	66	—	3	100	6
Hoffman & Goligher ('70)[10]	99	OPD	4	97	12
Notaras ('71)[9]	73	—	3	100	10
Miller ('71)[11]	99	OPD	2	100	5
Ray et al ('74)[12]	21	First B.M.	4	100	5
Oh ('74)	100	First B.M.	3	99	5
Rudd ('75)[13]	200	OPD	2	99.5	0.5

either by posterior open sphincterotomy (7) or lateral sphincterotomy (8–13). Comparative studies have demonstrated the superiority of lateral sphincterotomy over the posterior open sphincterotomy (10). (See Tables I and II.)

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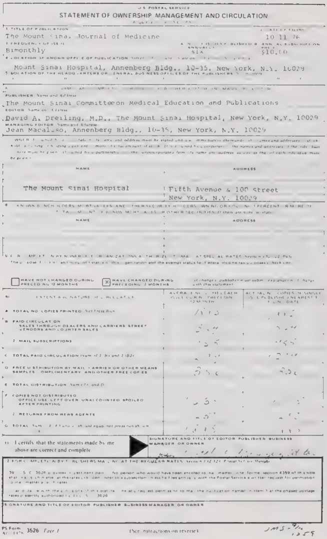

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CLINICAL CORRELATES OF DIAGNOSTIC MICROBIOLOGY
Part I

FESTSCHRIFT IN HONOR OF S. STANLEY SCHNEIERSON, M.D.

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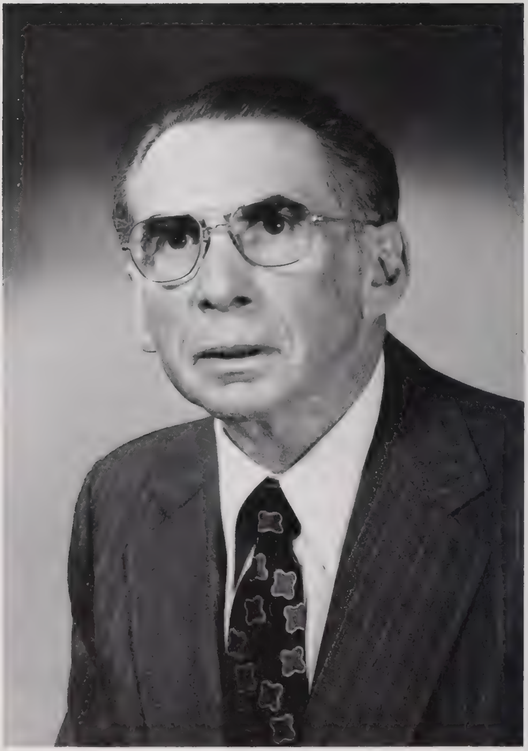

S. STANLEY SCHNEIERSON, M.D.

S. Stanley Schneierson, M.D.—An Appreciation

S. Stanley Schneierson, M.D. was born May 15, 1906 in the lower east side of New York City. He attended Boys High School in Brooklyn and was graduated from Cornell College receiving the A.B. degree in 1928. His first interest in college was forestry, but after one year Dr. Schneierson decided to make medicine his career. Following graduation from the Long Island College of Medicine in 1932 he interned at the Metropolitan Hospital on Welfare Island.

Dr. Schneierson entered private practice and began to work in the outpatient department of The Mount Sinai Hospital in 1933. Actually, Dr. Schneierson's association with Mount Sinai began much earlier in his life. Dr. George Baehr, who was Chief of Medicine at Mount Sinai, had been treating Dr. Schneierson's father for lung abscess. The young boy idolized Dr. Baehr and to a great extent it was the influence of Dr. Baehr that made Dr. Schneierson decide upon a career in medicine. It was also because of Dr. Baehr that Dr. Schneierson came to Mount Sinai. In 1934, while supporting himself in private practice, Dr. Schneierson began to help the late Dr. Gregory Shwartzman, then Director of the Microbiology Laboratory at The Mount Sinai Hospital, with his investigations of the Shwartzman phenomenon. Four years later Dr. Shwartzman arranged for Dr. Schneierson to spend a year in research with Dr. Maurice Weinberg in the Department of Anaerobic Bacteria at the Pasteur Institute in Paris. Before returning to the United States Dr. Schneierson also spent two months with Dr. Jules Bordet at the Pasteur Institute in Brussels and three months at the London School of Tropical Medicine and Hygiene. He returned to the United States to work with Dr. Shwartzman, but was inducted into the Navy in 1941. He served with the Marines as the Venereal Disease Control Officer in the Caribbean and later supervised a hospital and laboratory in Saipan. There Dr. Schneierson gained much experience in practical parasitology. He was discharged from the Navy in 1946 with rank of Commander. At this point Dr. Schneierson decided that he did not want to return to the practice of private medicine and became Dr. Shwartzman's assistant. When Dr. Shwartzman became ill in 1955 Dr. Schneierson was named Acting Director of the Microbiology Laboratory of The Mount Sinai Hospital and was appointed as Director in 1960. In 1966, with the establishment of Mount Sinai School of Medicine, Dr. Schneierson became the first Professor of Microbiology at the new medical school.

There is no doubt that Dr. Gregory Shwartzman exerted a profound influence on Dr. Schneierson's life. It was because of Dr. Shwartzman that Dr. Schneierson found his niche in medical microbiology. Indeed, while he was Dr. Shwartzman's assistant, Dr. Schneierson handled all the clinical problems and questions that came to the microbiology laboratory of The Mount Sinai

Hospital. Therefore, it was natural for Dr. Schneierson to become interested in the new field of antibiotics, to which he made many important early contributions. Together with Dr. Shwartzman, Dr. Schneierson first showed that antimicrobial agents such as sulfanilamide had no effect on bacterial toxins, but rather affected the bacteria themselves. During the 1940's Dr. Schneierson contributed many studies to elucidate the mechanism of action and clinical use of the then new wonder drug, penicillin. As more antimicrobial agents appeared, Dr. Schneierson developed quantitative methods for their study in the microbiology laboratory. Of note is the rapid disc-tube method of determination of bacterial sensitivity to antibiotics, a method that is now being used in automated systems for the determination of bacterial susceptibility to antibiotics. With the advent of computers, Dr. Schneierson quickly realized the applicability of electronic data processing to the clinical microbiology laboratory. In 1964 he described a punch-card system for identification of bacteria, now being used in commercial systems for computer identification of bacteria. In 1967 he described a punch-card system for recording, filing and analyzing antibiotic sensitivity patterns at The Mount Sinai Hospital on a monthly basis. The importance of such antibiotic sensitivity data was rapidly realized across the country and many hospitals now employ this system. Indeed, this type of data tabulation is now being offered commercially as a service to hospitals without their own computer facilities.

The Diagnostic Microbiology Laboratory of The Mount Sinai Hospital underwent tremendous growth and innovation with Dr. Schneierson's leadership. In 1955 Dr. Schneierson established a routine diagnostic virology laboratory, one of the first such laboratories in this country. This laboratory was an outgrowth of a research effort, in collaboration with Dr. Shwartzman, on the effect of corticosteroids on experimental polio infection. Together with the late Dr. Max Littman, Dr. Schneierson set up a separate mycology service laboratory. Indeed, in collaboration with Dr. Littman, the hazard of Cryptococcus neoformans infection in pigeon droppings in New York City was described. A serology laboratory was established with Drs. Jack Singer and Erwin Oreskes. Noteworthy, the latex fixation test was developed in this laboratory.

It is apparent that Dr. Schneierson's contributions have encompassed both clinical infectious diseases and the application of diagnostic mirobiology to the solution of clinical problems. Therefore, it is fitting that this festschrift entitled "Clinical Correlates of Diagnostic Microbiology" be dedicated to Dr. Schneierson in appreciation for his distinctive contributions to medical microbiology and his long years of dedicated service to The Mount Sinai Hospital.

Shalom Z. Hirschman, M.D.

Curriculum Vitae

S. Stanley Schneierson, M.D.
Born—May 15, 1906—U.S.A.
Education
  Cornell University—A.B.—1928
  Long Island College of Medicine—M.D.—1932
  Internship—Metropolitan Hospital, New York City—1932-1933
Training
  Research Assistant—Services of Dr. M. Weinberg and Dr. P. Grabar (Anaerobic Bacteriology and Chemistry)—Pasteur Institute—1938-1939
  Medical Mycology—College of Physicians and Surgeons—Dr. Rhoda Benham—1938
  Diplomate—Parasitology and Tropical Medicine—London School of Hygiene and Tropical Medicine, London—1939
  Research Assistant—Service of Dr. Jules Bordet (Immunology)—Pasteur Institute Brussels—1939
  Laboratory Diagnosis and Venereal Disease Control—Johns Hopkins University—1942
  Techniques of Radioactive Isotopes in Biology—Oak Ridge Institute of Nuclear Studies—1950
Hospital Appointments
  The Mount Sinai Hospital, New York, N.Y.
    Research Assistant in Bacteriology—1935-1938
    Assistant in Bacteriology—1938-1941
    Assistant Bacteriologist—1941-1945
    Associate Bacteriologist—1946
      (Title changed to Associate Microbiologist in 1952)
  The Mount Sinai Hospital, New York, N.Y.
    Attending Microbiologist and Acting Director of Microbiology—1955
    Director of Microbiology—1960—Emeritus—1974
    Consulting Microbiologist—Elmhurst General Hospital—1964
    Professor of Microbiology, Mount Sinai School of Medicine—January 1966—Emeritus—1974
Appointments and Memberships
  Diplomate—
  American Board of Pathology (Clinical Microbiology)—1950
  American Board of Microbiology (Public Health and Medical Laboratory Microbiology)—1962
  Fellow—
    New York Academy of Medicine—1947
    American College of Physicians—1947
    New York Academy of Sciences—1948
    American Association for the Advancement of Science—1951
    American Academy of Microbiology—1962
  Membership—
    Society for Experimental Biology and Medicine
    American Society for Microbiology
    American Association of Pathologist and Bacteriologists
    American Association of Immunologists
    American Society of Tropical Medicine
  Honorary Appointments—
    Councillor—N.Y. City Branch—American Society for Microbiology
    Member, N.Y. City Commissioner of Health's Advisory Committee on Clinical Laboratories
    Vice Chairman, N.Y. City Commissioner of Health's Board of Examiners for Clinical Laboratories
  Listed—
    American Men of Science

Military Service

U.S. Navy—January 1941–March 1946 (Epidemiology and Laboratory Director)
Rank—Commander

Chairman—Committee on Control of Infections—The Mount Sinai Hospital—1965–
Chairman—Committee on Laboratories—The Mount Sinai Hospital—1968–

S. Stanley Schneierson, M.D.

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Streptococcus: The Evolutional Parasite

LAWRENCE J. KUNZ, PH.D.

Introduction

The genus Streptococcus, a surprisingly complex bacteriologic taxon, seems to be accommodating very nicely in its parasitism of the species Homo sapiens despite a variety of relevant ecologic changes that have occurred over the last century.

Some of these changes seem certainly to have affected the champion parasite of the genus, Streptococcus pyogenes, the well-known Group A beta hemolytic streptococcus. Certain of the changes are inexplicable; others have been effected through the directed intervention of the bacterium's rational, concerned host, man.* Still others represent an adaptation between host and parasite. Overall, the reflecting student of nature—microbiologist, physician, or biology watcher—may discern, if he wishes, a shift among the component members of the genus Streptococcus that may well mark an evolutionary trend in parasitism by the genus vis-à-vis one of its many animal hosts, Homo sapiens.

That this host-parasite relationship of streptococci is only one of the ecologic niches filled by the bacterial genus in the animal kingdom cannot be overemphasized; on the contrary, it is important for the understanding of human streptococcal infections that appropriate perspective be applied to appreciation of the roles played by various streptococcal species in the colonization and infection of various human and subhuman members of the animal kingdom, particularly domestic animals. It is the purpose of this review to attempt to elucidate the various relationships referred to and to limn out an evolutionary path that might be anticipated for the genus over the next several decades in relation to human disease and medical progress.

Taxonomy and Nomenclature

Nomenclature applied to many of the species of streptococci testifies to the extensive, early contributions made by scientists concerned with dairy and veterinary bacteriology to the discovery and understanding of various members of the genus. This may be discerned from Table I which lists the species

From the Department of Microbiology and Molecular Genetics, Harvard Medical School; and The Francis Blake Bacteriology Laboratories, The Massachusetts General Hospital, Boston, Massachusetts.

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* The term "man" is used for simplicity in place of Homo sapiens with the implication that it refers both to the male and female of the species. Nothing demeaning or unflattering is intended by the deliberate omission of the term "woman."

TABLE I

*Generally Accepted Relationship of Lancefield Serologic Groups to Streptococcal Species**

Serological group	Streptococcal species
A	Str. pyogenes
B	Str. agalactiae
C	Str. equi, Str. zooepidemicus, Str. equisimilis, Str. dysgalactiae
D	Str. faecalis, Str. faecium, Str. bovis, Str. equinus
E	Str. infrequens, (Str. uberis?)
F	Str. minutus, Str. anginosus
G	Str. canis, Str. anginosus†
H	Str. sanguis (syn. Str. s.b.e.)
K	Str. salivarius
N	Str. lactis, Str. cremoris

* Adapted from Duma et al (1).

† Str. anginosus applies only to Type I Group G.

designations associated with the principal serologic groups (1); the predominance of names derived from animal and dairy terminology is remarkable. It is perhaps also an unfortunate commentary on motivation in medical research in that certain infectious diseases that affect both man and lower animals are occasionally controlled first in animals and then, if at all, in man. One speculates pessimistically that economic losses attending epidemic disease in marketable livestock or their products should have provided impetus for eradication of animal infections while humans, of no great immediate economic value, continued to be afflicted with the similar or comparable diseases. To be sure, elimination of carriers, enforced therapy and other factors which might have violated individual, personal human rights precluded widespread methods of control applicable to lower animals; yet continued prevalence of certain infectious diseases in man after their control in animals suggests less than adequate deployment of public health resources. The most distressing and astonishingly long-lasting effect of the veterinary terminology, however, is the persistent association, by occasional physicians, of so-named streptococci with infections of animals and an apparent reluctance to believe that "animal pathogens" could cause disease in their patients.

The taxonomy and nomenclature of streptococci outlined in Table I is perhaps oversimplified. For example, serological Group K streptococci are not completely coincident with the species, Streptococcus salivarius. Indeed the majority of nonhemolytic streptococci, i.e., of alpha or viridans streptococci and of gamma streptococci, are nongroupable serologically; their interrelationships are still being studied and no taxonomic schema for these organisms has been derived that has enjoyed a consensus. These streptococci are nonetheless, as will be seen presently, important pathogens of man in certain circumstances.

Reluctantly, it must be said that it is hardly possible any longer to retain for the pneumococcus the familiar and almost cherished binomial, Diplococcus pneumoniae. Our British colleagues have suffered our penchant for the term these many years. Streptococcus pneumoniae is the now inescapably valid name for pneumococci; and it will be used more frequently as our more recently trained colleagues join our ranks with more appropriate up-to-date scientific vocabulary. Clarification must be made of the occasional ambiguity with which the terms, enterococcus, fecal streptococci, Group D streptococci, and the like are occasionally used and of the irrelevance of the designations alpha, beta, and gamma hemolysis. These matters will be dealt with presently.

Pathogenicity

Allusion has been made to substantial changes that seem to have affected S. pyogenes as a human parasite. Three changes may be worthy of mention in this review for purposes of invoking an evolutionary thrust by the genus. Firstly, there must be mentioned the apparent decline in the virulence of S. pyogenes over the last century in respect to the degree of its pathogenicity. Thus, the mortality from scarlet fever in England and Wales declined from 982 per million living inhabitants in 1861–65, to half that twenty years later, to 29 per million during 1921–25, to zero mortality in 1960 (2). Maximum morbidity in recent years has occurred in the 5–10 year age group.

Secondly, the introduction of penicillin as a therapeutic agent to which S. pyogenes is exquisitely sensitive wreaked a devastating human-directed blow at the actual, if not potential, virulence of the organism. Thus, although severe epidemics of streptococcal disease still do occur even in moderately developed areas of the world (3), individual cases diagnosed early can be treated, and epidemics once they are recognized can be aborted by appropriate use of penicillin.

The third development relating to the genus Streptococcus that may have affected its evolutionary thrust was made in the collective mind of the medical profession with the recognition of the association of rheumatic fever and acute glomerulonephritis with prior infection of the patient by S. pyogenes. This intellectual event resulted in an unfortunate side-effect, an overwhelming preoccupation with Group A beta-hemolytic streptococci on the part of many individuals—concerned researchers, diagnostic bacteriologists and practicing physicians—almost to the exclusion of other, non-Group A streptococci from consideration as causative agents of significant disease in man. Expressions of this attitude are reflected in dissertations on streptococci in textbooks and monographs (4, 5) in which scant attention is given to human infections caused by streptococci other than S. pyogenes.

All in all, these three factors—natural decline in virulence, discovery of an efficacious antibacterial drug and concentrated effort on prophylaxis and treatment of infections—have led to a steady and significant decline in parasitism of man by the arch-parasite of the genus, S. pyogenes.

Meanwhile, streptococci other than S. pyogenes were all but neglected as human pathogens except for passing reference to streptococcal endocarditis and enterococcal infections in chapters in textbooks and lectures in microbiology and for occasional case reports in the literature of infections with one or another non-group A streptococci. It came as a surprise to many that Feingold's initiative to review nonrespiratory streptococcal infections of man at the Massachusetts General Hospital (6) resulted in recognition of an extraordinarily large number of infections of many body sites by streptococci other than S. pyogenes. Thus, of 173 infections attributed to streptococci, only 33.5 % were due to S. pyogenes; the balance, 115, were due to 10 other groups and to serologically ungroupable members the genus. Incidently, this study done in 1963 reflected fairly closely the findings of Foley (7) and of Rantz (8) some twenty years previously. A detailed analysis of streptococcal bacteremia at the Massachusetts General Hospital (1) revealed that many of the infections caused by non-group A streptococci occurred in patients with other, underlying diseases and often were associated with therapeutic measures (surgery, steroids, cytotoxic drugs, or antimicrobial therapy) intended to alleviate the underlying conditions.

From these observations there emerges the picture of a bacterial genus accommodating to the exigencies of a relationship with a host (formerly exquisitely susceptible to a single streptococcal species, S. pyogenes)—the host-species once again susceptible in his advancing years, with declining strength and diminished defenses, to less pathogenic but, perhaps, more exploitive, opportunistic members of the genus. That the present status of the struggle may not be entirely in favor of Homo sapiens should cause concern that the final outcome remains a matter worthy of our interest. At any rate, the genus Streptococcus vis-à-vis Homo sapiens is accommodating either evolutionally or by phenotypic adaptation and at present appears to be maintaining the status quo with its rationally antagonistic mammalian host.

Bacteriologic Diagnosis

The development of criteria and methods for recognition, identification and, later, differentiation of streptococci evolved through: description of reactions of colonies on blood agar media—so called alpha, beta and gamma "hemolysis" (9); biophysical and biochemical reactions of cultures (10); Lancefield's serologic grouping (11); and the particular application of the fluorescent antibody (FA) technique to recognition of Lancefield's grouping antigens in appropriately treated organisms. The FA method has been used extensively to recognize Group A streptococci (12) and, more recently, the increasingly notorious Group B organisms because of their association with neonatal meningitis (13). Use of the terms alpha, beta and gamma provide information of little or no clinical usefulness; the laboratory reactions that they denote may serve to guide the bacteriologist at the bench in initial steps in differentiation of streptococci present in a culture. Reservations have been expressed about the application of these terms both in performing bacterial examinations and in evaluating their results (1, 5).

The study of Duma and colleagues (1) previously cited not only confirmed the broad representation of streptococcal species in human infections found by Feingold (6) but also emphasized the serious nature of the infections. As can be seen from Table II the mortality of patients with bacteremia associated

TABLE II

*Mortality Associated with Bacteremia Caused by Various Serological Groups**

	Streptococcal groups					
	A	B	D	H	NG†	All groups (Including NG)
No. of patients with bacteremia	19	7	26	5	18	75
No. of deaths related to streptococcal infection	5	3	11‡	2	3‡	23§
Mortality	26%	43%	42%	40%	17%	31%

* Adapted from Duma, et al. (1).

† NG = Nongroupable with Lancefield antisera for Groups A through S.

‡ One patient included in this figure has a simultaneous Group D and nongroupable streptococcal bacteremia.

§ The patient with a simultaneous Group D and nongroupable streptococcal bacteremia is counted only once in this figure.

with streptococci of serological groups other than Group A was in most groups higher than that of patients with Group A streptococcal bacteremia. Because of this important fact and because of possible epidemiologic implications of the presence of certain streptococcal groups, a concerted effort has been made more definitively to identify streptococci associated with human infections. FA techniques were found to be applicable, but with qualifications, to several serological groups other than A (14). Further studies, however, suggested by observations of FA-stained cells, led to a procedure (15) for extracting easily and rapidly grouping antigen from all streptococci from primary isolation plates by the use of a mixture of Streptomyces albus extract (Maxted) and lysozyme. Cultures suitable for identification by the Lancefield precipitin technique that do not readily yield antigen by this method are readily grouped using the same easily applied mixture of enzymes for extraction of the antigen from a larger cell crop.

The easy applicability of serological grouping makes identification of most hemolytic strains of streptococci a matter of small expenditure of time, effort and expense. Two large groups of streptococci, however, require more time and effort for differentiation and identification to levels of clinically significant usefulness. For one of these, the Group D streptococci, methodology has been developed for identification of constituent members; for the other, adequate differentiation on a practical basis is still being investigated.

Group D streptococci comprise a somewhat heterogeneous congerie of incompletely related organisms. They have been extensively studied by many workers (16–20) and the importance of differentiating enterococci from nonenterococcal Group D species has been emphasized. One schematic representation of the taxonomic and colloquial designations is offered in Table III. Briefly, enterococci, relatively resistant to the penicillins and requiring synergistic combinations of antibiotics for effective therapy ought to be easily and accurately separated from the relatively antibiotic-sensitive S. bovis (20). One set of reactions for accomplishing this is presented in Table IV.

TABLE III

*Relationship of the Terms, Enterococci, Fecal Streptococci and Group D Streptococci**

	FECAL STREPTOCOCCI	
GROUP D STREPTOCOCCI	S. faecalis	ENTEROCOCCI
	S. faecium	
	S. durans	
	S. bovis	VIRIDANS STREPTOCOCCI
	S. equinus	
	Other streptococcal species	

* Adapted from (20) and others.

A large group of streptococci exist in an as yet unresolved melange of biochemical, morphologic and serologic incompatibilities. These are the so-called ungroupable (although sometimes serologically reactive) alpha or nonhemolytic streptococci. They are variously referred to as "mouth flora," "pharyngeal flora," "viridans streptococci," "nonhemolytic streptococci," or by some other, pejorative term denoting questionable parentage. Valiant efforts have been made by many investigators (21, 22, 23) to redistribute and assemble these streptococci of dubious parentage into the format of a respectable taxonomic grouping. One possible solution to the problem likely to be accepted by clinical bacteriologists is the system of grouping and nomenclature devised by Colman and Williams (23); it employs more or less conventional bacteriologic methods for differentiation and recognition of the respective, proposed species. As these authors indicate, their system is not incompatible with that of Carlsson (22); and if workers from two separate laboratories can begin to agree on the relatedness of 5 or 6 groups of so diverse a potpourri of organisms as the "nonhemolytic streptococci" they must be doing something right! I look forward to testing this conclusion, especially since Colman and Williams include an old friend, D. pneumoniae, among six species proposed in their working proposal, viz., S. pneumoniae, S. salivarius, S. mitior, S. milleri, S. sanguis, and S. mutans.

Aberrant Strains

One cannot deal effectively with the streptococci from an evolutionary viewpoint without at least some slight reference to aberrant forms of the organism. The magnitude of the clinical importance of cell-wall-deficient strains of various species of streptococci will remain a matter for speculation until exhaustive comprehensive studies are performed by several groups of workers that might elucidate the frequency of occurrence and circumstances surrounding their involvement in cultures of clinical specimens. In addition to

TABLE IV

*Reactions to Various Tests Used for Speciation of Group D Streptococci**

Test	Enterococci		S. bovis
	S. faecalis	S. faecium	
Growth on 40% bile	+	+	+
Esculin hydrolysis	+	+	+
Growth on 6.5% salt broth	+	+	–
Starch hydrolysis	–	–	+
Arginine hydrolysis	+	+	–
Resistance to 60°C for 30 min	+	+	V
Gelatin hydrolysis	V	–	–
Resistance to tellurite	+	–	–
Tetrazolium reduction	+		V
Production of extracellular polysaccharide on 5% sucrose agar	-	–	V
Clot in litmus milk	+	–	+
Acid from			
Lactose	+	+	+
Sucrose	+	+	+
Mannitol	+	+	V
Sorbitol	+	–	V
Arabinose		+	V
Raffinose	–	V	+
Salicin	+	+	+
Melibiose	–	+	+
Melezitose	+		–

* Adapted from (20).

Note: + = positive reaction; – = negative reaction; V = variable, some strains positive, others negative.

cell-wall-deficient variants of streptococci, biochemically deficient "satelliting" strains of streptococci have been reported with increasing frequency in the last several years. These strains have required either pyridoxine (24) or thiol-containing compounds (25).

From reports cited it would appear that the thiol-requiring streptococci seem to have survival value in vivo where they seem to have been implicated in both acute and subacute infections. The inference has been drawn that cell-wall-deficient organisms may provide a protected life-state between recurrences in illness, in infectious diseases marked by temporary cure and subsequent relapse upon cessation of antimicrobial therapy. Whether or not these phenomena are evolutionary must remain highly speculative. Nevertheless, they do demand the attention and reactivity of the microbiologist if their involvement in disease is to be detected and the extent of the involvement to be assessed with any degree of accuracy.

Summary and Conclusions

A speculative inquiry was made of the genus Streptococcus with respect to any possible evolutionary trend and pathogenetic potential that might be developing in relation to one of its mammalian hosts, Homo sapiens. It was tentatively concluded that some species of the genus had indeed changed in one direction or another in respect to pathogenicity vis-à-vis man, but that the genus as a whole was at least maintaining the status quo pathogenetically, if not assuming a superior evolutionary stance in respect to and in spite of its sole rational host.

Certain practicable, clinically relevant information and data have been provided to adjust to or compensate for any inaccuracy of the conclusions drawn from factual data cited.

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The Quellung Reaction, A Neglected Microbiologic Technique

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> The thing that hath been is that which shall be; and that which is done is that which shall be done: and there is nothing new under the sun.
> *Ecclesiastes* i, 9

There are few techniques available to the diagnostic microbiologist that rival the quellung reaction for ease of performance, speed of results, accuracy, and economy. In conjunction with the Gram stain, it may permit the prompt identification of several bacterial species as components of the microbial flora of man and, when such organisms are isolated from some bodily sites, establish them as the cause of illness. These facts notwithstanding, the quellung reaction is employed with relative infrequency today. The reasons for its neglect are several, and they will be cited in the brief historial review of the technique and of its application which follows. It is hoped that these considerations may stimulate a return to the more general use of the reaction by diagnostic microbiologists.

The quellung reaction resulting from the interaction of bacterial capsular polysaccharide with homologous anticapsular antibody was discovered by Franz Neufeld and reported by him in a paper entitled: "Ueber die Agglutination der Pneumokokken und über die Theorie der Agglutination" published in 1902 in the *Zeitschrift für Hygiene und Infektionskrankheiten* (1). He described the reaction with rabbit antiserum in the following fashion:

> If one mixes equal parts of agglutinating serum and of pneumococcal broth culture, be it in a test tube or by emulsifying a loop of serum and of culture on a coverglass, one observes in the hanging drop a significantly swollen appearance which develops immediately or in several minutes with a powerfully effective serum. The single cocci swell to double and triple their size, there is flattening of their points of contact which are otherwise tapered in relation to one another and their contours become indistinct and blurred. Meanwhile, let it be recognized that in this quellung process, chiefly the outer layers of the bacterial cell are concerned; they convert themselves into a homogeneous glassy mass while in the middle of the coccus a dark round nucleus is to be seen. The circumstance gives next the impression that, through the action of the serum, a high grade degeneration and the beginning of the solution of the bacterium takes place. This impression is strengthened by the fact that such swollen pneumococci have

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> lost their stainability. When dried and fixed in the usual fashion, they take up almost no dye; if one adds cautiously a dye to the fluid drop, then one succeeds at most in staining the dark kernels described in the middle of the actually swollen masses, which themselves remain uncolored.

Application of this important observation to diagnostic microbiology was not to take place until nearly three decades later; for, at the time it was made, there existed few indications of the serologic heterogeneity of pneumococcus. The reaction was cited again in 1912 by Neufeld and Händel in the second edition of Kolle and Wasserman's *Handbuch der pathogenen Mikroorganismen* (2) and depicted in two figures for the first time. Although two years earlier, these same workers had established clearly the existence of two discrete serotypes of pneumococcus (3), they apparently saw then no application of the quellung reaction to the serotyping of the organism.

In the same year, Aoki, working in Strasbourg, described what was undoubtedly a manifestation of the quellung reaction (4). The technique he employed to demonstrate the phenomenon differed somewhat from the conventional one, but his results are nonetheless of historic interest. Following admixture and incubation of the sediment of a pneumococcal broth culture with either control or homologous horse antiserum, he made air-dried preparations on slides which were stained with 1% aqueous methylene blue and counterstained with dilute carbol fuchsin. By the application of this technique, he reached a number of interesting conclusions regarding the interaction of pneumococcus with an homologous antiserum which are hereby quoted (4):

> 1) In vitro, pneumococci form thicker and more beautiful capsules in immune serum and in immune serum containing fluids than in normal serum.
> 2) One can also observe this phenomenon in vivo. When one injects with living pneumococci a rabbit which has been pretreated with immune serum, the pneumococci surround themselves with a fine thick capsule.
> 3) Capsule formation proceeds more rapidly at body temperature than at lower temperature.
> 4) Dead pneumococci form just as fine capsules as living ones.
> 5) They (capsules) form in inactivated serums as well as in fresh serums.
> 6) The formation of the capsule is not a vital reaction but a physicochemical one.

The foregoing observations include much of what is known about several aspects of the quellung reaction.

Considerable progress was made in the second and third decades of this century in the delineation of pneumococcal capsular types. Extending the work of Neufeld and Händel, Dochez and Gillespie defined clearly three pneumococcal types by immune reactions and assigned the remaining strains to a heterogeneous "Group IV" (5). Analogous work was carried out in South Africa by Lister, who, in 1917, identified 11 discrete serotypes (6). By 1929, Cooper and her associates in New York had described 32 capsular types of pneumococci (7). In all this work, the techniques used to establish the diverse types of pneumococcus had included mouse protection tests, precipitin tests, macroscopic and microscopic agglutination tests and bactericidal tests with whole blood, but not the quellung reaction.

The third edition of Kolle and Wasserman's handbook appeared in 1928 and again the chapter on pneumococcus was written by Neufeld, this time in collaboration with Schnitzer (8). The quellung reaction is cited therein once more, together with microphotographs of the organism following exposure to homologous antiserum and to control serum. The cells were stained with dilute crystal violet. The authors seemed reluctant to recommend microscopic agglutination with homologous antiserum in a hanging drop preparation as a means of typing pneumococci, however, lest false agglutination resulting from aggregation of organisms at the drying margins of the droplet lead to misinterpretation of the results. In the same chapter, the photograph of the refractile hyaline precipitate formed by the union of type 2 pneumococcal capsular polysaccharide with homologous anticapsular rabbit serum from the paper of Schiemann and Casper (9) is reproduced. It has the same optical properties as the capsular material surrounding the pneumococcal cell following its interaction with type specific anticapsular serum.

The first application of the quellung reaction to the identification of pneumococci was described by Neufeld and Etinger-Tulczynska in 1931 in a paper on nasal pneumococcal infection and carriage by mice, rabbits and guinea pigs (10). Nasal swabs of inoculated animals gave rise usually to mixed bacterial cultures in their experiments. They were able, nonetheless, readily to identify pneumococci, as is indicated by the following quotation from their report: "However, one can quickly and surely identify pneumococci if one makes use of the following fact, that they swell (quellen) markedly immediately in homologous rabbit serum (not horse serum!). One mixes on a coverglass a loop of serum bouillon culture with a loop of rabbit immune serum and observes in a hanging drop. It is very easy (also with the dry system) to recognize the markedly swollen, almost yeastlike appearing pneumococci. The absence of these forms in control droplets clinches the diagnosis." Two photographs show clearly the presence of quelled pneumococci in microscopic preparations of mixed cultures. A footnote contains the following comment: "In addition, moreover, the quellung reaction has proved simpler and more dependable for the type diagnosis of pneumococci than agglutination."

Meanwhile, the clinical application of the quellung reaction to the typing of pneumococci from patients with pneumonia was described for the first time by Armstrong in England in the same year (11). He used an indirect method, inoculating mice intraperitoneally with sputum and examining their peritoneal exudate four hours later. Droplets of the latter were mixed with rabbit antisera of types 1, 2 or 3 or with normal rabbit serum on a glass slide, covered with a cover slip, and examined unstained. "The immediate result, in the case of a positive reaction, is a conspicuous increase in the size of the pneumococci due to conjugation of coccus and homologous antibody, Brownian movement is retarded and the pneumococci become very apparent as lanceolate bodies surrounded by a clear, highly refractive capsular zone." He was aware of Neufeld's work as indicated by the following statement: "Neufeld, twenty years ago, applied the microscopic method to typing pneumococci, but was dissatisfied with the results he obtained with Type III"; but he did not cite Neufeld in his list of references.

The following year, Armstrong (12) and Logan and Smeall (13), in consecutive papers in the British Medical Journal, reported the application of rabbit antisera to the direct typing of pneumococci in sputum.

A major contribution to the understanding of the quellung reaction was published by Neufeld's collaborator, Etinger-Tulczynska, in 1932. Entitled "Bacterialkapseln und 'Quellungsreaktion'" (14), the report cites the following attributes of the phenomenon: "The reaction is strongly type specific and has the following advantages over agglutination and precipitation: 1. Prompt appearance in one minute; 2. Economy in the use of immune serums; 3. Applicability also in mixed cultures; and 4. Directly in blood, organ juices, exudates and sputum." The interpretation of the reaction is stated succinctly in the following quotation: "Accordingly, the quellung reaction is identical in its essence to precipitation; only in the first case it involves carbohydrate formed in the capsule, in the second case, free (carbohydrate)."

Etinger-Tulczynska reported also the occurrence of the quellung reaction with klebsiellas (Friedländer's bacillus), streptococci and anthrax. The paper includes the following conclusion: "A *specific quellung reaction* following the addition of homologous serum will be observed only with those of the above cited bacteria which are *capsulated*. The essence of the reaction lies in the fact that the carbohydrate in the capsule precipitates and the previously invisible capsule is made visible thereby; it can also swell considerably."

Success in typing pneumococci in sputum is cited in the same report; and, in June of the following year, Neufeld and Etinger-Tulczynska published a brief paper entitled: "Rapid Diagnosis of Pneumococcal Types from the Sputum" (15) in which they described identification of the organism in sputum of 14 of 16 cases by the quellung reaction.

The publication which had probably the greatest impact on the introduction into the United States of the quellung technique for the direct typing of pneumococci in sputum was that of Sabin, which appeared in the Journal of the American Medical Association of May 15, 1933 (16). Sabin described learning the technique from Kenneth Goodner, who had visited Neufeld's laboratory at the Robert Koch Institute in Berlin and who had used the technique himself. Among the important details stressed concerning the method was the use of rabbit rather than of equine antisera in the performance of the test. Sabin studied 100 cases of pneumonia at Bellevue Hospital and identified pneumococci in 76. Among them were included 31 strains of type 1, 22 of type 2, and 4 of type 3. Types 4, 5, 7, 8 and 13 were also recognized.

Sabin's report had immediate impact. The employment of pooled antisera to 3 to 10 pneumococcal types, suggested for use in Sabin's earlier slide agglutination test (17) by Bullowa and Schuman (18) in 1930, was applied to the quellung reaction by Cooper and Walter, who reported also that pneumococci in sputum could be typed readily after two weeks' refrigeration (19). Beckler and MacLeod described in 1935 the results of examining 760 sputum specimens for the typing of pneumococci by the Neufeld method and found it to be the most rapid and sensitive technique for this purpose in a study carefully controlled by the use of other techniques (20).

Over the next decade, during which serum therapy of pneumococcal pneumonia was employed, albeit it with declining frequency following the introduction of sulfonamides in 1938, the quellung reaction was widely employed as the principal method for the identification and typing of pneumococci. In the same period, the number of recognized pneumococcal types continued to be augmented chiefly by Mørch in Denmark (21) and by Eddy (22) in the United States. Identification of pneumococci by the quellung technique was facilitated markedly by the introduction in 1963 of pneumococcal "Omni-serum," a serologic reagent containing concentrated rabbit antibodies to all 82 pneumococcal types known at that time (23).

Despite the foregoing advances in the identification and typing of pneumococci, their application was to be short-lived. The introduction of penicillin for the treatment of pneumoccal pneumonia by Tillet and his coworkers in 1945 (24) led to the abandonment of serum therapy, with its requirement for knowledge of the capsular type of the invading pneumococcus; and the importance of identifying pneumococcal types responsible for human illness became less apparent to physicians. In addition, typing serum, which had been a by-product of therapeutic serum, soon ceased to be available commercially in the United States. With the abandonment of pneumococcal typing and, concomitantly, with the discard of the technique of inoculating sputum intraperitoneally into the mouse for the isolation of pneumococci, recognition of the organism declined strikingly. As a result thereof, the view that pneumococcal pneumonia had declined significantly in frequency came to be one held rather widely by physicians in the sixth decade of this century. Reintroduction of the classic methods for the isolation and identification of pneumococci, however, has suggested, in several studies, that there has been little if any change in the attack rates of pneumococcal pneumonia in this country and abroad, whenever appropriate bacteriologic methods have been employed.

The technique of the quellung reaction is a simple one. Although several modifications are known, the following procedure has proved highly satisfactory in the writer's hands (25). A loopful of emulsified sputum, body fluid, mouse peritoneal washings or liquid culture is spread over an area 0.5 to 1 cm in diameter on a glass slide and allowed to dry at room temperature. In similar fashion, the cells from a single colony of pneumococcus may be emulsified in a drop of sterile broth or of normal salt solution on a slide and allowed to dry. A loopful of 1 % aqueous methylene blue is placed on a cover slip, and a loopful of typing serum is then applied directly to the dried spot on the slide. The residual antiserum adhering to the loop is next mixed with the methylene blue on the cover slip and then applied to and mixed with the droplet of antiserum on the slide which has been spread previously over the area of the dried preparation. The cover slip is inverted over the wet preparation and blotted lightly. A drop of immersion oil is applied to the cover slip and the preparation is examined under the oil immersion lens of a microscope with 10× ocular lenses.

For best results, it is essential to employ oblique illumination; a microscope with a fixed substage lamp will be unsuitable for this purpose. The light from an illuminator should be reflected by a substage concave mirror through the condensor so that only the left lower third of the microscopic field is brightly illuminated when viewed through a low power (10×) objective. The other factor of importance is the density of the pneumococci in the preparation. It should be limited to 50 to 100 organisms per oil immersion field. A greater number of pneumococci may result in a prozone phenomenon when homologous anticapsular serum is applied, with inhibition of the quellung reaction by the excess antigen in the preparation. This phenomenon is most likely to be encountered with pneumococci of types 3 and 37 because of the large amount of capsular polysaccharide produced by each.

Identification of individual pneumococcal types is facilitated by the combination of monospecific antisera into pools of three or more type-specific anticapsular sera. The quellung reaction is carried out with successive pools until a positive reaction is observed. The antisera to individual types comprising the pool are tested then until a type-specific reaction can be identified. Because more than one capsular type of pneumococcus may be present in a single sputum specimen (as many as five have been recognized), it is important to observe whether or not all organisms resembling pneumococci react with a given antiserum. If not, then testing with the remaining pools of antiserum should be carried out. Although uncommon, simultaneous infection of the middle ear and of the blood with two pneumococcal types has been described.

The availability of pneumococcal "Omni-serum" (23, 26) provides the physician with an invaluable reagent for the rapid identification of pneumococci. This reagent can be used not only for the recognition of pneumococci in respiratory secretions but also for the rapid diagnosis of pneumococcal meningitis, empyema, arthritis and peritonitis. The presence of pneumococci in such body fluids can be established in a matter of minutes by use of the quellung technique, and it should be employed as a routine diagnostic procedure in all hospitals for this purpose. It has become evident also that, if the diagnosis of putative pneumococcal pneumonia is to be made with a reasonable degree of accuracy, the use of the quellung reaction is required. In the current program to redevelop pneumococcal vaccines sponsored by the National Institute of Allergy and Infectious Diseases, the recognition of putative and of proved pneumococcal infection in populations under scrutiny has risen 2- to 10-fold following reintroduction of the quellung technique as a routine diagnostic procedure. Analogous results have been reported recently also from a university hospital (27).

The quellung reaction can be utilized to facilitate the identification and typing of other bacteria pathogenic to man. The occurrence of the phenomenon with Group A (Type I) meningococci was reported in 1935 by Clapp et al (28), and extended later by others to organisms of Group C (29, 30). Group B meningococci, however, fail to give a quellung reaction. Alexander used the technique for the identification of H. influenzae type b in 1939 (31) and reported its application to the six known capsular types of this organism in 1946 (32). Described first by Etinger-Tulczynska (14), the quellung reaction has become the principal method for the typing of klebsiellas (33). Strains of several species of streptococci may produce capsules of carbohydrate, including those of Lancefield's Groups B (34) and F (35), Streptococcus salivarius (36), and many strains of alpha and nonhemolytic streptococci. A number of these organisms can be recognized by the quellung reaction (37, 38). Although strains of Group A beta hemolytic streptococci may produce a capsule of hyaluronic acid, this polysaccharide is not antigenic in mammals; and the quellung reaction cannot be used to identify it.

The quellung reaction may be manifested also by bacteria of the genus Bacillus which are endowed with capsules of polypeptide. This phenomenon was described first by Etinger-Tulczynska (14) and elucidated by Tomcsik (39, 40).

The interaction of a fungus with homologous rabbit antiserum to give what may have been a quellung reaction was reported by Roger in France in 1897 (41), five years before its description with pneumococcus by Neufeld in Germany (1). Roger immunized rabbits intravenously with repeated sublethal doses of live oïdium (Candida) albicans. When the organism was inoculated into serum, it grew poorly and in compact masses. When an aqueous suspension of cells from such a culture stained with methylene blue was examined microscopically, its appearance was described in the following fashion: "Let us first envisage an isolated yeast: the protoplasm is colored as in the normal state: it has the same appearance and dimensions. Only it is surrounded by a colorless mass, hyaline, somewhat lightly striated, the borders of which are sinuous, poorly delimited, of which the size is five to ten times larger than that of the normal cuticle. The elements are rarely isolated . . . . It is easy to convince onself that it is not a matter of simple juxtaposition but a fusion of the cuticles, of the formation of a true zooglea." The same phenomenon was observed when cells from a colony of the organism were mixed directly on a slide with antiserum and examined microscopically. The two drawings in the report, one of cells grown in normal serum and the other of cells grown in immune serum present a marked contrast, the latter resembling strikingly the quellung reaction. Analogous reactions with other fungi have been reported in this century, including those with Cryptococcus neoformans and Sporotrichum schenckii (42, 43).

The nature of the quellung reaction has been the subject of considerable study and debate as to whether or not the phenomenon involves true swelling of the capsule (44, 45). Regardless of how one interprets the several experimental findings, it is clear that the combination of antibody with a capsular polymer outside the microbial cell wall gives rise to changes in the capsule's optical properties which are readily identifiable through the microscope. In addition, it makes possible anatomic localization of the bacterial antigen taking part in the reaction.

The specificity of the quellung reaction is essentially no different from that of other antigen-antibody reactions; and it is possible, at times, by means thereof to identify cross reactions between different capsular types of the same species and similar reactions among organisms of different bacterial genera and species. Cross reactions among types of pneumococci are not uncommon (21, 22) and serve as a basis for the grouping of closely related serotypes in the diagnostic sera prepared by the Danish Statens Seruminstitut (46). Many strains of alpha and nonhemolytic streptococci produce capsules which cross react with one or another of 28 different pneumococcal serotypes (38, 47) and those of H. influenzae (48), klebsiellas (49) and E. coli (50) possessing analogous properties have been recognized as well. Although in most instances these cross reactions are unlikely to lead to confusion when the quellung reaction is employed for diagnostic purposes, they make essential employment of the Gram strain and of cultural techniques to confirm the identity of a given organism recovered in the course of an illness.

The quellung reaction requires significant levels of antibody to become manifest, and it is seldom a useful technique for the detection of specific antibodies in the serum of man. It can be employed on occasion, however, to provide confirmation of the diagnosis of bacterial endocarditis or endarteritis, when serum of the patient obtained at the time bacteremia was demonstrated gives a positive quellung reaction with the organism isolated from the blood culture.

Reaction of capsular polymers with proteins other than antibodies may give rise to optical phenomena similar to those of the quellung reaction (51) and are of interest though they play no role in diagnostic microbiology. C reactive protein, a globulin appearing in the serum of man in a variety of inflammatory states, may give rise to the quellung reaction in the presence, but not in the absence, of calcium ions with a variety of bacterial capsules composed of phosphorylated polysaccharides (52, 53). In addition, Shin et al (54) have used a modification of the quellung phenomenon to demonstrate the attachment of the third component of complement (C3) to a pneumococcal capsule. A positive reaction followed incubation of cells of pneumococcus type 25 in fresh guinea pig serum, washing of the cells and then treating them with specific antibody to C3.

As implied by the foregoing remarks, the quellung reaction has considerable potential utility and requires only the simplest of laboratory equipment. In conjunction with the Gram strain, it provides a rapid and reliable technique for the identification of three important human pathogens: pneumococcus, H. influenzae and klebsiellas, when capsulated variants of any of these species gives rise to infection in man. Second, it permits identification of serotypes of several bacterial species, some of which have greater invasive powers than others of the same species. Information of this kind has utility in assessing the potential pathogenic role of an organism in a given illness when found coexisting with other organisms in secretions such as sputum, and it has also some prognostic value. Third, identification of those serotypes of a bacterial species manifesting a high degree of invasiveness and causing frequent infection plays an important role in the selection of capsular antigens for inclusion in prophylactic vaccines. Finally, serotyping of capsulated bacteria by means of the quellung reaction provides an epidemiologic tool for the identification of bacterial strains, such as those of klebsiellas, giving rise to outbreaks of nosocomial infection (55).

Conclusion

Because of the simplicity, speed and economy of the quellung reaction, it is the writer's hope that the foregoing comments will stimulate microbiologists to make greater use of its potential than has been made in the recent past and to assure, by their concerted action, that the necessary reagents for its accomplishment will become generally available to diagnostic laboratories once again. I have known and enjoyed Stanley Schneierson for almost two decades. I think he will share my enthusiasm for the quellung reaction. Although it may be lacking in novelty, it remains a most useful and elegant bacteriologic technique.

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Pseudomonas Species in Clinical Microbiology

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Abstract

Unusual or infrequently encountered Pseudomonas species in clinical bacteriology are reviewed. Criteria for identification of pseudomonads and related forms, their antibiograms, as well as their ecology, incidence, and clinical significance are discussed. Although pseudomonads are saprophytes and contaminate human clinical specimens, these bacteria may serve as opportunistic pathogens under certain conditions, and those factors predisposing to infection are summarized.

Introduction

Clarification of nomenclature and development of new bacteriologic methods have resulted in improved criteria for identification of Pseudomonas species in clinical microbiology. Of the many Pseudomonas species recovered from human clinical materials, only three (P. aeruginosa, P. pseudomallei, P. mallei) are usually thought of as associated with disease in man. The remaining pseudomonads were considered as rarely isolated saprophytes or contaminants of clinical specimens. However, the increase of opportunistic infections caused by these infrequently encountered pseudomonads has led to greater medical interest in their role as potential human pathogens.

A number of recent reports have documented both the characteristics of pseudomonads (1–13) and infections caused by unusual Pseudomonas species (14–17). This report presents the author's data on the morphologic features, biochemical characteristics, and antimicrobial susceptibilities of pseudomonads, as well as a literature review of the incidence and clinical significance of the species encountered. P. aeruginosa, P. pseudomallei, and P. mallei (the latter two, the causative agents of melioidosis and glanders, respectively) are excluded from the review because their clinical significance has been repeatedly documented in the literature.

General Characteristics of Genus Pseudomonas

Major Features Used for Genus Determination

Pseudomonads are Gram-negative, asporogenous, straight or slightly curved, unicellular bacilli. Motile species are polarly flagellated. Either they do not attack carbohydrates or utilize them oxidatively with the production of acid but no gas. Energy is not obtained by fermentative or photosynthetic

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metabolism. Since pseudomonads are obligate aerobes, molecular oxygen is the universal hydrogen acceptor; some species, however, respire in the presence of nitrate and grow under anaerobic conditions in the presence of this substrate or arginine as an electron acceptor. All species are catalase-positive and give negative reactions for indole, methyl red, and acetylmethylcarbinol production. With certain exceptions, the species are strongly positive for indophenol oxidase.

Pseudomonads are not fastidious in their growth requirements. They grow well on nonselective media such as nutrient and blood agars and often develop on most selective-differential agar media. Deoxycholate Agar and Salmonella-Shigella Agar may be inhibitory for some species. The majority, but not all isolates will develop on MacConkey Agar. Most species grow on a basal mineral medium, without growth factors, containing ammonium ion as sole source of nitrogen and glucose as sole source of carbon and energy. A few species require amino acids and vitamins as growth factors.

Differential Diagnosis from Other Nonfermentative Bacteria

A pseudomonad which conforms to the above description will produce nonlactose-fermenting colonies on enteric differential agar media. The same pseudomonad will show no visible growth or a neutral pH in the butt of Kligler Iron Agar or Triple Sugar Iron Agar with either an alkaline, neutral, or in the case of P. pseudomallei and rare isolates of other species, an acid pH in the slant. However, other nonfermenters display similar reactions on these media and must be differentiated from Pseudomonas. Several major groups of nonfermenters are recognized on the basis of cellular morphology, oxidase activity, and motility (flagellar arrangement): polar monotrichous or polar multitrichous (Pseudomonas) and peritrichous (Alcaligenes, Bordetella bronchicanis, Achromobacter), oxidase-positive bacilli; atrichous, oxidase-negative diplococci (Acinetobacter) and oxidase-positive diplobacilli (Moraxella); and atrichous, oxidase-positive, yellow-pigmented bacilli (Flavobacterium). Acinetobacter, Moraxella, and Flavobacterium are easily distinguished from Pseudomonas since the former are nonmotile. The immotile species P. mallei is identified by certain biochemical features (Tables I and III). Examination of flagellar anatomy differentiates the peritrichously flagellated Alcaligenes, B. bronchicanis, and Achromobacter from Pseudomonas. In some instances it is more expedient to establish the identity of these bacteria with the same battery of biochemical tests used for the characterization of pseudomonads.

Tests and Media Used for Identification

The following tests are sufficient for identification of Pseudomonas species. Unless otherwise stated, all cultures are incubated aerobically at 30°C for 24 to 48 hr. At this temperature many reactions including acid production from carbohydrates and pigmentation develop faster than at 35°C. In addition some isolates grow slowly at 35°C.

Morphologic Studies

Smears prepared from the 24-hr growth on Trypticase Soy Agar (TSA; BBL) are stained by Gram's method (Hucker's modification) and then observed for microscopic morphology. Motility is determined by microscopic examination of a hanging drop preparation of a Trypticase Soy Broth (BBL) culture. To demonstrate flagella, Gray's method as outlined by Bailey and Scott (18) is used. Accumulation of poly-beta-hydroxybutyrate (PBHB) is determined by Sudan black staining (19) of cultures grown on mineral base medium supplemented with DL-beta-hydroxybutyrate used for carbon assimilation studies (5).

Isolated colonies on TSA plates containing 5% (w/v) of defibrinated sheep blood (TSBA) are examined for morphology and hemolytic activity. Growth characteristics are also observed on Salmonella-Shigella Agar (SS Agar; BBL), MacConkey Agar (BBL), and Deoxycholate Agar (DC Agar; BBL). Fluorescein (pyoverdin) production is detected by exposing cultures on Sellers' Differential Agar (Difco), Pseudosel Agar (BBL), or Pseudomonas Agar F (Difco) to UV irradiation. Pyocyanin production is determined through the use of Pseudomonas Agar P (Difco).

Physiologic Studies

A standard inoculum consisting of one 4 mm loopful of a 24-hr TSB culture is used for most tests. Growth from a TSA or TSBA culture is used for the patch method of inoculation and for inoculating the gluconate oxidation and ortho-nitrophenyl-beta-D-galactopyranoside (ONPG) tests.

Oxidation of carbohydrates. Oxidation of 1% (w/v) solutions of carbohydrates is tested in OF Basal Medium (OFBM; Difco) and oxidation of 10% (v/v) of lactose is tested in Purple Agar Base (Difco).

Gluconate oxidation. Production of 2-ketogluconate is detected by adding a loopful of growth and a gluconate substrate tablet (Key Scientific Products) into a tube containing 1.5 ml of sterile water. After incubation for 24 hr the culture is tested for reducing substances by adding a Clinitest tablet (Ames Co.) and observing for a green or orange color indicative of a positive reaction.

ONPG test. Beta-D-galactosidase is detected by adding a loopful of growth to Beta-Galactoside Test Broth (Hyland) and observing for the formation of a yellow color after overnight incubation.

Nitrate reduction. Reduction of nitrate to nitrite is determined as outlined by Bailey and Scott (18) using Trypticase Nitrate Broth (BBL) plus 1.5% agar. Ability to grow anaerobically in the presence of nitrate or nitrite is indicated by the development of a blue color in the butt of Sellers' medium. Nitrogen gas production is ascertained in the two former media by the production of gas bubbles in the condensate as well as in the agar butts.

Urease, indole, and hydrogen sulfide production. Christensen's urea agar (BBL) and Kligler Iron Agar (KIA; BBL) or Triple Sugar Iron Agar (TSIA;

Difco) are used, respectively, for detection of urease and hydrogen sulfide production. Indole production is tested for by adding Kovac's reagent to SIM Medium (BBL) cultures after incubation for 24 hr.

Decarboxylase, dihydrolase, and deaminase activity. Phenylalanine deaminase activity is determined by flooding Phenylalanine Agar (BBL) slant cultures after 24 hr incubation with a 1% (w/v) ferric chloride solution and observing for the development of a green color. Arginine dihydrolase and lysine and ornithine decarboxylase activity are detected using Decarboxylase Base Moeller (Difco).

Production of extracellular enzymes. The patch method of inoculation is used for the following tests: starch hydrolysis is tested by flooding TSA plates supplemented with 1% (w/v) of starch with Lugol's iodine after 24 hr and observing for a clear zone around the patch of growth. The production of lipase is tested on TSA plates supplemented with 1% (w/v) of polyethylene sorbitan monooleate (Tween 80) and observing for a turbid halo around the growth. Lecithinase production is tested by observing for a white precipitate around the growth on a TSA plate supplemented with 10% (v/v) of Egg Yolk Enrichment (BBL). Liquefaction of gelatin is determined in nutrient gelatin. Casein digestion is determined using TSA plus 10% (v/v) of skim Milk Powder (BBL) and observing for a clear zone around the growth. Deoxyribonuclease (DNase) activity is detected by incorporating 0.01% (w/v) of toluidine blue into DNase Test Agar (Difco) and observing for a change in the indicator to pink. Esculin hydrolysis is tested by observing for a black precipitate around the growth on a TSA plate supplemented with 0.1% (w/v) of esculin and 0.05% (w/v) of ferric citrate.

Indophenol oxidase activity. Oxidase activity is detected by rubbing the growth from a TSBA culture onto an oxidase test paper strip (Patho Tec-Co). A dark reddish-purple color appears in 10 to 60 sec with oxidase-poitive strains.

Tolerance to chemical and physical factors. Growth at 42°C is determined using TSA slant cultures incubated at 42° ± 0.05°C. In the following tests the agar plates are streaked with a small inoculum: tolerance to 0.03% (w/v) of cetrimide is tested using Pseudosel Agar plates and to triphenyltetrazolium chloride (TTC) using TSA plates supplemented with 1% (w/v) of TTC. The ability to grow at pH 5.6 is tested on Sabouraud's Dextrose Agar. Salt tolerance is determined by adjusting the NaCl concentration of TSA plates to 2.5 and 6.5% (v/v).

Assimilation of organic compounds as a sole source of carbon. Growth at the expense of organic compounds is determined by utilizing a mineral base medium (MBM) containing (g/L): $(NH_4)_2SO_4$, 2.0; $NaH_2PO_4 \cdot H_2O$, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1. The respective carbon sources are added to the basal medium to give a final concentration of 0.03M (pelargonate, DL-norleucine) and 0.015M (acetate). Each medium is adjusted to pH 6.5 ± 0.1. The ingredients, excluding agar, made at double concentration are sterilized by filtration, and brought up to working concentration by adding the appropriate volume of sterile melted 3.2% agar. The mixture is then dispensed into sterile tubes and slanted.

Antimicrobial susceptibility. Strains are tested for antibiotic susceptibility according to the standardized single-agar disc diffusion method of Bauer and associates (20).

Schema for Identification of Pseudomonas Species

The results of physiologic and antimicrobial studies with 709 isolates are listed in Tables I and II, respectively, and represent an extension of data previously published (4, 21). Table III lists the salient features required for identification of Pseudomonas species. Since biochemical and nutritional tests that are time-consuming to perform or require long incubation periods are omitted, this schema can be implemented by most routine diagnostic laboratories that have a limited number of available substrates. The schema is reliable and will identify the majority of pseudomonads 24 to 48 hr after performance of the tests.

Discussion of Pseudomonas Species

Fluorescent Group (P. aeruginosa, P. fluorescens, P. putida)

Morphology and Physiology. Typical strains of P. aeruginosa produce pyocyanin, a water-soluble, nonfluorescent, blue, phenazine pigment that is soluble in chloroform. P. aeruginosa strains may synthesize various combinations of pyocyanin, pyoverdin (fluorescein), pyorubin, and pyomelanin (brown to black water-soluble pigment). Less than 3% of clinical isolates are pyomelanogenic (11). Whereas pyocyanin-producing strains of P. aeruginosa are easily identified in the laboratory, apyocyanogenic strains must be differentiated from other pseudomonads. Apyocyanogenic strains can be recognized by several uniform characters, namely polar monotrichous flagella, growth at 42°C, and failure to oxidize disaccharides in OFBM. Universal features of pyocyanogenic strains, such as nitritase, gelatinase, caseinase, and lipase activity, production of 2-ketogluconate, and triphenyltetrazolium tolerance, are unreliable for the identification of apyocyanogenic strains (4, 21).

P. fluorescens and P. putida (P. ovalis) possess polar multitrichous flagella, and most, but not all, isolates of these species produce pyoverdin and arginine dihydrolase. Pyoverdin is a water-soluble, yellow, fluorescent, pteridine pigment that is not soluble in chloroform. Pseudomonads encountered in clinical specimens which produce modified phenazine pigments, including P. chlororaphis, P. aureofaciens, and P. lemonnieri, are considered biotypes of P. fluorescens (5). Some strains of P. fluorescens and P. putida do not grow at 35°C; since clinical specimens are usually incubated at 35° or 37°C, their recovery rate is below what would be expected at lower incubation temperatures. P. fluorescens is distinguished from P. putida by gelatinase, caseinase, and lecithinase activity, and assimilation of select organic compounds as sole sources of carbon and energy. P. fluorescens and P. putida are differentiated

TABLE 1

Characteristics of Pseudomonads and Related Bacteria[a]

Test or substrate	*Pseudomonas aeruginosa* (61)	*P. fluorescens* (68)	*P. putida* (84)	*P. pseudomallei* (6)	*P. mallei* (6)	*P. cepacia* (62)	*P. acidovorans* (31)	*P. testosteroni* (5)	*P. alcaligenes* (21)	*P. pseudoalcaligenes* (18)	*P. stutzeri* (54)	*P. putrefaciens* (23)	*P. maltophilia* (238)	*P. diminuta* (6)	*Xanthomonas* (7)	Group VE-1 (10)	Group VE-2 (9)
Acid: Glucose, 1% (OFBM)	98	100	100	100	100[b]	100	97	0	0	94	100	87	100	0	100	100	100
Fructose	93	97	99	100	100	100	100	0	0	100	100	30	98	0	100	100	100
Galactose	89	99	100	100	100	100	0	0	0	6	98	4	39	0	100	100	100
Mannose	87	99	100	100	100	100	0	0	0	6	93	9	97	0	100	100	100
Rhamnose	28	77	61	83	0	0	0	0	0	0	61	13	0	0	0	80	89
Xylose	85	97	98	100	0	100	0	0	0	22	100	4	56	0	100	100	100
Lactose	0	27	27	100	100	98	0	0	0	0	0	13	90	0	100	0	0
Sucrose	0	60	14	83	0	84	0	0	0	0	0	26	93	0	100	0	11
Maltose	0	63	36	100	100	98	0	0	0	28	100	22	100	0	100	100	89
Mannitol	69	93	18	100	83	100	97	0	0	0	85	0	0	0	0	100	100
Lactose, 10% (PAB)	20	53	43	100	·	100	0	0	0	0	0	4	0	0	71	100	100
Gluconate oxidation	66	68	85	0	0	11	0	0	0	0	1	0	0	0	0	0	0
ONPG	0	3	1	0	·	84	0	0	0	0	0	0	95	0	100	100	44
Pyoverdin	87	94	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen sulfide (KIA)	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Urea	79	38	49	50	17	34	0	0	33	0	24	17	0	0	29	10	33
Nitrite production	34	6	0	83	·	27	100	80	48	100	61	100	41	0	0	100	0
Nitrogen gas production	61	3	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0
Indophenol oxidase	100	100	100	100	67	90	100	100	100	100	100	100	1	100	100	0	0
Arginine dehydrolase (DBM)	98	99	98	100	100	0	0	0	0	22	2	0	0	0	0	70	0
Lysine decarboxylase	0	0	0	0	0	94	0	0	0	0	0	0	99	0	0	0	0
Ornithine decarboxylase	0	0	0	0	0	47	0	0	0	0	0	100	0	0	0	0	0
Phenylalanine deaminase	2	3	0	0	·	0	0	2	24	33	37	0	0	0	0	0	56
Esculin hydrolysis	0	0	0	67	·	81	0	0	0	0	0	17	100	0	100	100	0
Lipase	67	41	2	83	·	100	16	60	48	0	94	100	100	0	71	0	44
Starch hydrolysis	0	0	1	0	·	0	0	0	0	0	89	0	0	0	57	50	56
Deoxyribonuclease	13	0	0	0	·	0	0	0	0	0	0	100	100	100	0	0	0
Lecithinase	11	97	0	100	·	31	0	0	0	0	0	0	0	0	0	0	0
Gelatinase	57	100	0	100	0	74	0	0	5	0	0	96	100	100	0	20	0
Caseinase	60	100	0	100	·	75	3	0	4	0	0	96	100	100	0	20	0
Hemolysis	46	21	1	50	·	7	0	14	0	0	0	0	0	0	0	0	0
Growth on SS Agar	89	97	99	0	67	3	81	0	0	78	85	87	0	0	0	50	11
Growth on MacConkey Agar	98	100	100	100	·	97	97	80	100	100	100	100	100	100	0	100	100
TTC-tolerance	77	81	75	0	·	31	3	0	14	11	0	0	0	0	0	0	0
6.5% NaCl-tolerance	5	4	12	0	·	0	0	0	0	6	100	96	0	0	0	70	44
2.5% NaCl-tolerance	100	99	100	0	·	47	42	20	52	100	100	100	100	100	43	100	100
pH 5.6-tolerance	95	99	98	100	·	100	100	100	100	56	0	0	100	100	100	80	100
Cetrimide-tolerance (PA)	95	97	88	0	·	77	3	0	10	61	0	0	0	0	0	10	22
Growth at 42 C	100	0	0	100	0	71	23	0	81	100	100	100	74	100	14	100	66
Wrinkled colonies	0	0	0	83	0	0	0	0	0	0	100	0	0	0	0	40	78
Growth in MBM + acetate	98	100	100	100	100	98	100	100	91	100	100	96	4	0	71	100	100
Pelargonate assimilation	100	100	100	100	·	100	0	0	100	100	13	0	·	·	0	100	100
DL-Norleucine assimilation	0	0	0	0	·	0	100	100	0	0	0	0	·	·	0	0	0
Motile	98	100	100	100	0	100	100	100	100	100	100	100	100	100	71	100	100

[a] Parenthetical values refer to number of strains examined; other values indicate percent of strains giving reaction. Except where indicated, the majority of reactions were interpreted after 24 to 48 hr of incubation at 30 C. All strains were indole-negative. *P. aeruginosa* strains were apyocyanogenic. Acid production from glucose by some strains of *P. acidovorans* and *P. pseudoalcaligenes* was weak. Acid production from carbohydrates by some strains of *P. putrefaciens* was delayed several days. Indophenol oxidase activity by some strains of *P. pseudomallei*, *P. mallei*, and *P. cepacia* was weak. Starch hydrolysis by Group VE strains was weak or equivocal. OFBM, OF Basal Medium; PAB, Purple Agar Base; ONPG, ortho-nitrophenyl-*beta*-D-galactopyranoside; KIA, Kligler Iron Agar; DBM, Decarboxylase Base Moeller; SS Agar, Salmonella Shigella Agar; TTC, triphenyltetrazolium chloride; PA, Pseudosel Agar; MBM, mineral base medium.

[b] Results of *P. mallei* are from Hugh and Gilardi (15).

from apyocyanogenic strains of P. aeruginosa on the basis of flagellar arrangement, growth at 42°C, and oxidation of disaccharides.

Sources of Isolation and Clinical Significance. The fluorescent pseudomonads are common inhabitants of soil and water. P. fluorescens and P. putida have been isolated from hospital water sources, benzalkonium chloride solution, fluid from an ultrasonic nebulizer, intravenous catheter, and contaminated blood and blood products stored in the refrigerator (11, 22). These species represent part of the normal oropharyngeal flora (23), and most strains are recovered in the clinical laboratory from respiratory tract specimens (24, 25). Other clinical sources include wounds, pleural fluid, urine, cerebrospinal fluid, skin lesion, bile drain, feces, and blood (17, 22–25). P. fluorescens and P. putida are usually environmental contaminants and rarely opportunistic pathogens for man. They have been associated with empyema (26), a febrile reaction following transfusion of contaminated blood (27), urinary tract infections (16, 28), abscesses (16), wound infections (16, 28, 29), septicemia (28–30), and septic arthritis (31). Both P. fluorescens and P. putida

TABLE II

Antimicrobial Susceptibilities of Pseudomonads and Related Bacteria[a]

Antibiotic	*P. aeruginosa* (61)	*P. fluorescens* (68)	*P. putida* (84)	*P. pseudomallei* (6)	*P. cepacia* (62)	*P. acidovorans* (36)	*P. alcaligenes* (39)	*P. stutzeri* (54)	*P. putrefaciens* (23)	*P. maltophilia* (238)	*P. diminuta* (6)	*Xanthomonas* (7)	Group VE (19)
Penicillin	0	0	0	0	0	0	13	13	13	4	0	0	0
Novobiocin	0	0	0	83	37	30	8	0	0	3	100	57	0
Erythromycin	5	4	12	0	5	11	56	65	87	9	100	100	100
Lincomycin	0	0	0	0	0	0	0	0	0	0	0	0	0
Ampicillin	8	0	0	83	0	3	23	89	52	13	0	43	100
Tetracycline	21	84	74	100	5	83	85	93	57	24	100	100	100
Chloramphenicol	15	44	30	83	97	89	59	61	96	88	100	100	100
Streptomycin	34	53	73	0	1	3	33	89	96	23	0	14	100
Cephalothin	0	3	1	0	0	6	28	2	26	1	100	0	0
Nitrofurantoin	3	0	2	0	0	56	13	2	100	0	0	0	0
Nitrofurazone	6	4	23	50	0	11	46	11	100	2	0	29	0
Nalidixic acid	41	16	51	83	81	94	80	93	100	91	0	43	74
Neomycin	70	94	99	83	53	11	64	100	100	32	100	71	100
Kanamycin	54	87	99	100	68	42	72	98	100	36	100	71	100
Polymyxin	98	99	100	0	0	75	92	100	100	94	0	29	100
Gentamicin	95	100	98	0	15	6	58	100	100	39	0	71	100
Carbenicillin	60	2	18	0	3	19	65	91	64	21	0	43	100

[a] Parenthetical values refer to number of strains examined; other values indicate percent of strains susceptible. *P. aeruginosa* strains were apyocyaongenic. *P. acidovorans* includes closely related *P. testosteroni* strains. *P. alcaligenes* includes closely related *P. pseudoalcaligenes* strains. This schema is not intended for use as an indicator of drug of choice for therapy and is presented only as a diagnostic guide for the identification of these pseudomonads. Strains were tested according to the standardized single-disc method of Bauer and associates (20).

TABLE III

Salient Features for Identification of Pseudomonads and Related Bacteria[a]

Morphology, test or substrate	*P. aeruginosa* (apyocyanogenic)	*P. fluorescens*	*P. putida*	*P. pseudomallei*	*P. mallei*	*P. cepacia*	*P. acidovorans*	*P. testosteroni*	*P. alcaligenes*	*P. pseudoalcaligenes*	*P. stutzeri*	*P. putrefaciens*	*P. maltophilia*	*P. diminuta*	*Xanthomonas*	Group VE-1	Group VE-2
Acid: Glucose, 1% (OFBM)	+	+	+	+	+	+	+^w	−	−	+^w	+	+/−^d	+	−	+	+	+
Fructose	+	+	+	+	+	+	+		−	+	+	−/+^d	+	−	+	+	+
Xylose	+/−	+	+	+	−	+			−	−/+	+	−	+/−	−	+	+	+
Lactose		−/+	−/+	+	+	+	−	−	−	−		−/+^d	+	−	+	−	−
Maltose	−	+/−	−/+	+	+	+	−	−	−	−/+	+	−/+^d	+	−	+	+	+/−
Mannitol	+/−	+	−/+	+	+/−	+	+		−	−	+/			−		+	+
Lactose, 10% (PAB)	−/+	+/−	−/+	+	·	+	−	−			−		·		+/−	+	+
ONPG	−	−	−	−	·	+/			−		−		+	−	+	+	/+
Pyoverdin	+/−	+	+/−	−	−	−	−	−	·	−	−	−	−	−	−		−
Hydrogen sulfide	−	−	−		−	−	−	−		−	−	+	−				
Nitrogen gas	+/−	−	−	+	−			−	−	−	+	−	−	−	−		−
Indophenol oxidase	+	+	+	+^w	+/−^w	+^w	+	+	+	+	+	+	−	+	+	−	−
Arginine dihydrolase	+	+	+	+	+	−	−	−	−	/+	−	−	−	−		+/	
Lysine decarboxylase		−		−	−	+	−	−	−		−	−	+	−	−		−
Ornithine decarboxylase		−		−	·	−/+	−	−	−			+		·	−	·	
Esculin hydrolysis	−			+/−	·	+/−		−				/+	+	−	+	+	−
Starch hydrolysis	−	−	−	−	·	−		−	·	−	+		−	−	+/−	+/−^w	+/^w
Deoxyribonuclease	−/+	−	−	−	·	−	−		−	·	−	+	+	+			−
Lecithinase	−/+	+		+	·	−/+	−	−	−		−		−	−	−	−	
Gelatinase	+/−	+		+		+/−	−	−	−	−		+	+	+	−	−/+	−
6.5% NaCl-tolerance		−	−/+	−		−		−	−	−	+	+	−	−	−	+/−	−/+
pH 5.6-tolerance	+	+	+	+	·	+	+	+	+	+/−		−	+	+	+	+/	+
Cetrimide-tolerance	+	+	+/−	−	·	+/−	−		−	+/−		−	−	−			−/+
Growth at 42 C	+		−	+	·	+/	−/+		+/−	+	+	+	+/−	+	/+	+	+/−
Polymyxin susceptible	+	+	+	−	−	−	+/−	+	+	+	+	+	+	−	/+	+	+
Growth in MBM + acetate	+	+	+	+	+	+	+	+	+	+	+	+		−	+/	+	+
Pelargonate	+	+	+	+	·	+	−	−	+	+	−/+		·	·	−	+	+
DL-Norleucine	−	−	·		·	−	+	+	−	−			·	·	−		
Wrinkled colonies		−	−	+/−	−	−	−			−	+	−	−	−	−	−/+	+/−
PBHB accumulation	−		−	+	+	+	+	+	−	−/+	−		−	+	·	·	·
No. of flagella	1	>1	>1	>1		>1	>1	>1	1	1	1	1	>1	1	1	>1	1

[a] −, 90% or more of strains negative; +, 90% or more of strains positive; −/+, 11–49% of strains negative; +/−, 50–89% of strains positive; w, weak reactions in some strains; d, reactions delayed several days in some strains; OFBM, OF Basal Medium; PAB, Purple Agar Base; ONPG, ortho-nitrophenol-*beta*-D-galactopyranoside; KIA, Kligler Iron Agar; DBM, Decarboxylase Base Moeller; PA, Pseudosel Agar; SS Agar, Salmonella-Shigella Agar; MBM, mineral base medium; PBHB, poly-*beta*-hydroxybutyrate; 1, polar monotrichous; >1, polar multitrichous; ·, not tested. Not all strains were examined for flagellar anatomy or PBHB accumulation; the results are based on the fraction of strains tested.

are generally susceptible to tetracycline, neomycin, kanamycin, polymyxin, and gentamicin (16, 22, 25, 32). In contrast to pyocyanogenic P. aeruginosa strains, P. fluorescens and P. putida strains are susceptible to kanamycin and tetracycline, and resistant to carbenicillin (22, 32). There is less contrast, however, between the antibiograms of nonpigmented P. aeruginosa strains and the P. fluorescens-putida complex.

Pseudomallei Group (*P. pseudomallei, P. mallei, P. cepacia*)

Morphology and Physiology. The phytopathogen P. cepacia (P. multivorans, P. kingii, EO-1), the cause of onion bulb rot, is genetically related to the animal pathogens P. pseudomallei and P. (Actinobacillus) mallei (33). The principal characters of the pseudomallei group include accumulation of PBHB as cellular reserve material, polar multitrichous flagella, and utilization of a wide spectrum of organic compounds as carbon and energy sources (34). Many strains fail to produce or produce a slow and very weak indophenol oxidase reaction. The form and color of the colonies of these species are highly variable, ranging from rough to smooth in texture and gray to yellow in color.

Motility, growth at 42°C, denitrification, and oxidation of xylose separate P. pseudomallei from P. mallei. P. mallei grows relatively slowly on media when compared with P. pseudomallei, and the cell crop yield is lower (11). Although P. pseudomallei may demonstrate variation in colonial morphology, most strains have wrinkled, rough, corrugated colonies. P. cepacia shares phenotypic similarities with P. pseudomallei and P. mallei, but tests for ONPG, denitrification, arginine dihydrolase, and lysine decarboxylase separate P. cepacia from the latter species. P. cepacia is usually motile, but immotile isolates have been encountered (11). This species grows well at 30°C, but some strains grow poorly at 35°C. Some isolates produce a yellow-green, water-soluble, nonfluorescent, phenazine pigment on various media; a few strains produce a brown, red, or purple pigment (5). The pigments may diffuse into the agar medium or remain confined to the colonies. Pigment synthesis is variable, and many strains are nonpigmented.

Sources of Isolation and Clinical Significance. P. cepacia has a wide geographic distribution and has been isolated from distilled water, disinfectants, detergent solutions, saline solution, anesthetics, thermometer, respirators, intravenous fluid, rotting tree trunk, soil, and natural, river, and tap water (11). It is frequently recovered from wet surfaces and water in the hospital environment (35, 36) and has been isolated from a wide variety of clinical specimens including urine, wounds, blood, respiratory tract, synovial fluid, ear, and toe webs of troops with "foot rot" (11, 37). P. cepacia has been associated with septicemia (36, 38–40), endocarditis (40–45), urinary tract infections (37, 46–48), pneumonia and pneumonitis (37, 49–51), and various abscesses and wound infections (16, 35). P. cepacia is resistant to gentamicin and antibiotics of the polymyxin group. Only chloramphenicol has been shown to be effective against this species (15, 17, 52). Successful treatment of P. cepacia endocarditis with trimethoprim-sulfamethoxazole has been reported

(43–45). The antibiogram of P. cepacia is similar to those of P. pseudomallei and P. mallei (53).

Acidovorans Group (P. acidovorans, P. testosteroni)

Morphology and Physiology. Günther (54) gave the first description of a strain belonging to this taxon, which he named Vibrio terrigenus. The specific epithet terrigenus has been transferred to several genera and various names have been used for this species including Vibrio percolans, Lophomonas alcaligenes, Pseudomonas acidovorans, Pseudomonas testosteroni, and Comamonas percolans. Hugh (6) proposed Comamonas terrigena as the type species, and only species in the genus Comamonas. A neotype strain, ATCC 8461, for Comamonas terrigena, was designated (6) which has the characteristics which Günther attributed to the species. Hugh (7) includes P. acidovorans and P. testosteroni as a segment of C. terrigena. Stanier, Palleroni, and Doudoroff (5) differentiate the two species on nutritional characteristics and consider the specific epithet terrigena as a synonym for acidovorans.

Members of the acidovorans group are polar multitrichous and accumulate PBHB. The flagella are distinctive, resembling those of spirilla. The wave length of the polar flagella averages 3.10 μm with an average amplitude of 1.18 μm (55). Generally negative physiological features are associated with members of the acidovorans group. As a consequence, it can be confused with the nutritionally similar strains of the alcaligenes group. The acidovorans group (and the alcaligenes group) contain in part species which are weakly saccharolytic; these species oxidize fructose, but oxidation of glucose may be weak, equivocal, or not at all. P. acidovorans, but not P. testosteroni, forms acid from fructose, mannitol, and usually glucose. P. testosteroni was named on the basis of its ability to grow on testosterone and related steroids (5).

Sources of Isolation and Clinical Significance. P. acidovorans and P. testosteroni have a wide geographic distribution, being recovered from soil, water, and animal sources (11). They have been isolated from human sources including blood, abscess, urine, wound, and respiratory tract, but no clinical significance has been associated with these isolates. Strains of the acidovorans group are similar to strains of the alcaligenes group, but differ from other pseudomonads in that no single antibiotic is uniformly effective against them (52).

Alcaligenes Group (P. alcaligenes, P. pseudoalcaligenes)

Morphology and Physiology. Members of this relatively inert group are separated from the physiologically similar strains of the acidovorans group by polar monotrichous flagella and assimilation of appropriate organic compounds. Pelargonate, but not norleucine, supports growth of members of the alcaligenes group. P. pseudoalcaligenes is nutritionally more active than P. alcaligenes; it may accumulate PBHB, and it oxidizes fructose and usually glucose, but not mannitol, and may form acid from several other carbohydrates. P. alcaligenes biotype B appears to correspond to P. pseudoalcaligenes (5, 11).

Sources of Isolation and Clinical Significance. P. alcaligenes and P. pseudoalcaligenes have been isolated from inhalation therapy equipment, raw milk, and various animal and water sources (11), and in man from blood, urine, respiratory tract, and abscesses. They are rarely opportunistic infectious agents. P. alcaligenes has been associated with empyema (15). P. pseudoalcaligenes was the etiologic agent of pneumonitis and a postoperative knee infection (16) and was isolated from an infection of a pregnant uterus (56). No single antibiotic is uniformly effective against members of the alcaligenes group (52).

Stutzeri Group (P. stutzeri, P. mendocina)

Morphology and Physiology. Members of this nonfluorescent group are polar monotrichous, denitrify, and tolerate 6.5% NaCl. The majority of freshly isolated strains of P. stutzeri (Bacillus denitrificans II, P. stanieri, Group Vb) produce colonies that are both wrinkled, tough, and coherent as well as smooth. Most strains form a buff to light-brown intracellular pigment. The ability to denitrify tends to be lost by many strains after repeated subculture. The marked nutritional heterogeneity of this species is demonstrated by the relatively small number of organic compounds that serve as substrates for all strains (5). Most, but not all strains will grow on maltose and starch, the latter being attacked by extracellular amylase.

P. mendocina is differentiated from P. stutzeri on the basis of positive arginine dihydrolase but not amylase activity and in not utilizing maltose (57). Colonies of P. mendocina are flat and smooth and contain a pale-yellow carotenoid pigment. P. mendocina can be confused with denitrifying biotypes of P. fluorescens; however, the former is polar monotrichous, and fails to produce pyoverdin, gelatinase, and lecithinase.

Of major importance is the differentiation between P. stutzeri and P. pseudomallei since these species may produce similar rough yellow colonies. However, they may be separated on the basis that P. pseudomallei, but not P. stutzeri, oxidizes lactose and sucrose, produces gelatinase and arginine dihydrolase, and fails to grow on SS Agar.

Sources of Isolation and Clinical Significance. P. stutzeri is ubiquitious in soil and water and has been found in manure, humus, straw, sewage, dung, stagnant water, baby formula, animals, aerosolization equipment, inanimate surfaces, and eye cosmetics (11, 17, 58). The organism lives as a saprophyte in man and has been recovered from numerous clinical specimens (58), but only on few occasions has this pseudomonad been directly associated with an infectious process, e. g., otitis media, an old osteomyelitic lesion of the tibia, and wounds sustained after trauma (16, 59). P. stutzeri belongs to the relatively antibiotic-susceptible group of pseudomonads and frequently demonstrates susceptibility to ampicillin, tetracycline, streptomycin, nalidixic acid, neomycin, kanamycin, polymyxin, gentamicin, and carbenicillin (15–17, 32, 59). P. mendocina is found in soil and water but apparently is infrequently recovered from clinical specimens.

P. maltophilia

Morphology and Physiology. P. maltophilia (P. melanogena) strains are polar multitrichous, and produce lysine decarboxylase and extracellular deoxyribonuclease. P. maltophilia is unusual in not producing indophenol oxidase (8). This pseudomonad appears to lack cytochrom *c*, which may be necessary for the indophenol oxidase reaction (5). Methionine is an essential growth factor (5). When grown on agar media, P. maltophilia produces a water-soluble, pale-yellow pigment which does not diffuse into the medium.

Sources of Isolation and Clinical Significance. P. maltophilia has a wide geographic distribution being found in water, soil, and vegetable sources. It has been isolated from raw milk, sewage, tissue culture, frozen fish, feces of various animals and reptiles, rotten eggs, streptomycin solution, disinfectants, soil in petroleum areas, and the hospital environment (11). Common human sources include respiratory tract, wounds, blood, and urine, but P. maltophilia is recovered from almost any site on or within the body (17, 60). P. maltophilia is occasionally an opportunistic pathogen for man. It has been associated with acute mastoiditis (61), endocarditis (62), bacteremia (14), pneumonia (14), urinary tract infections (29, 63), and various wound infections and abscesses (15, 16, 63). This species is resistant to many antibiotics, with most strains demonstrating susceptibility only to chloramphenicol, nalidixic acid, and polymyxin (15–17, 32).

Diminuta Group (P. diminuta, P. vesicularis)

Morphology and Physiology. Strains of this group are polar monotrichous, accumulate PBHB, and produce indophenol oxidase. The flagella are unusual, having a wavelength of only 0.6 to 0.98 μm (64). Nitrate cannot be used as a nitrogen source. Since the strains of this group have complex growth factor requirements, none of them can grow in an MBM with organic compounds as sole carbon and energy sources (65). P. diminuta (Group 1-a) requires cystine, pantothenate, biotin, and cyanocobalamin for growth. It fails to oxidize carbohydrates but some strains form acid from ethanol (64). P. vesicularis is distinguished from P. diminuta by utilization of glucose, galactose, and maltose and production of carotenoid pigment (2, 65). Its growth factor requirements include the above mentioned vitamins but not cystine.

Sources of Isolation and Clinical Significance. P. diminuta has been isolated from water, tissue culture, and inhalation therapy equipment (11, 17). It has been isolated from human sources but appears to have no etiologic significance. P. diminuta strains are susceptible to chloramphenicol but resistant to polymyxin and gentamicin. Apparently P. vesicularis is infrequently recovered in nature or clinical specimens from man.

P. putrefaciens

Morphology and Physiology. P. putrefaciens (P. rubescens, Group 1-b) is a polar monotrichous bacillus that produces hydrogen sulfide and blackens the stabbed butt of KIA or TSIA; for this reason strains may be mistaken for Salmonella. The ability to produce hydrogen sulfide is lost by some strains on subculture (17). Ornithine decarboxylase and extracellular deoxyribonuclease are produced. Some strains grow poorly or fail to grow at 37°C. Oxidation of carbohydrates is variable; production of acid from glucose may be prompt, slow, or not at all. A reddish-tan or pink water-soluble pigment is formed on most media.

Sources of Isolation and Clinical Significance. P. putrefaciens has a wide geographic distribution, being found in soil and water. It has been recovered from dairy products, eggs, snake feces, sewage, stagnant and river water, tissue culture, oil emulsion, and natural gas and petroleum brines. It is responsible for spoilage of food including butter, haddock fillets, and fresh meat (7, 11). P. putrefaciens has been isolated from various human sources (11) but has been etiologically associated only with otitis media (16, 66) and a tibia wound infection (16). This species belongs to the drug-susceptible group of pseudomonads demonstrating susceptibility to 10 of 17 antibiotics tested.

Species Related to Pseudomonas: Xanthomonas and Group VE

Xanthomonas (Group II-k) is the second genus in the family Pseudomonadaceae, but on the basis of DNA homology and DNA base composition studies (67), it has been proposed that xanthomonads be placed in the genus Pseudomonas as a single genetic species P. campestris. Although xanthomonads are polar monotrichous, motility can not always be detected. They form a yellow, carotenoid-like pigment soluble in alcohol but not water (68). Many strains fail to produce or produce a slow and very weak indophenol oxidase reaction. Xanthomonads utilize many carbohydrates but fail to oxidize polyhydric alcohols. Most, but not all strains produce a copious slime when grown on agar media containing glucose or a number of other carbohydrates. Xanthomonads are primarily phytopathogens with a worldwide distribution. They have been isolated from the hospital environment (17) and from clinical specimens of human origin (15) without any association with disease. Xanthomonas is susceptible to chloramphenicol, tetracycline, and erythromycin and variably susceptible to other antibiotics (17).

Weaver, Tatum, and Hollis (13) applied the designations VE-1 and VE-2 to groups of oxidative bacilli which form a water-insoluble, yellow pigment and which resemble Xanthomonas in many respects. Bacteria with similar features were previously designated Chromobacterium typhiflavum (1). Members of these groups lack indophenol oxidase and utilize a wide range of organic compounds as carbon and energy sources. Their colonies may be wrinkled, tough, and coherent. Group VE-1 strains are polar multitrichous, and produce positive tests for arginine dihydrolase, esculin hydrolysis, and nitritase activity. Group VE-2 strains are polar monotrichous and negative for these same biochemical features. Group VE strains are differentiated from Xanthomonas by oxidation of mannitol but not lactose. Strains of these groups have been recovered from blood, urine, and sputum without any association with infection (15). Members of these groups are relatively drug-susceptible, responding to 11 of 17 antibiotics tested.

Summation of Clinical Significance of Infrequently Encountered Pseudomonads

The pseudomonads examined in this report appear to be free-living and ubiquitous and have both an endogenous habitat in the human body and an exogenous habitat in water, soil, or on plants. Although they are frequently saprophytes or contaminants, these organisms may serve as primary or opportunistic pathogens. They have a low degree of virulence with limited invasiveness for normal human hosts, but with impaired host defenses these pseudomonads can produce extensive suppurative lesions. Septicemic symptoms associated with these bacteria are attributed to endotoxin release.

An examination of the cases documented in the literature indicates that some of the manifestations of infection with pseudomonads are associated with certain predisposing factors. There is an increased incidence of infection in elderly patients and in patients who have had serious underlying medical problems such as burns, trauma following surgery, slow-healing wounds, narcotic addiction, malignancy, or other major debilitating conditions. Infections may be of nosocomial origin in patients who have had prolonged hospitalization, associated with a history of prior surgical intervention, intravascular instrumentation, or manipulative procedures, such as urethral catheterizations, cystoscopies, tracheostomies, lumbar punctures, and intravenous infusions of medications and fluids. Hospital-acquired infections also occur in patients who have been placed on long-term therapy with immunosuppressive agents, corticosteroids, antimetabolites, and antibiotics. Radiation therapy also predisposes to infection by these species. Prompt diagnosis of pseudomonas infections, identification of the species involved, and determination of specific therapy are necessary for a favorable prognosis.

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Detection of Unusual Strains of Gram-Negative Rods through the Routine Use of a Deoxyribonuclease-Indole Medium

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Among the various commercial systems used for the identification of Gram-negative rods, only one includes a test for extracellular deoxyribonuclease (1). In this laboratory, a medium for the simultaneous testing of deoxyribonuclease (DNase) activity and indole production using tubed DNase Test Agar with Methyl Green (Difco Laboratories, Detroit, Michigan) has been employed since 1973 for the routine identification of nonlactose fermenting Gram-negative rods (2). Inclusion of this medium into a test battery has led not only to the rapid identification of Serratia marcescens–S. liquefaciens, Proteus vulgaris, Aeromonas hydrophila, and Pseudomonas maltophilia but also to the detection of eight unusual strains belonging to the species Vibrio parahaemolyticus, P. putrefaciens, and S. rubidaea whose characteristics and clinical relevance will be discussed here.

Materials and Methods

During the 15-month observation period (June, 1973 to September, 1974) this laboratory used Desoxycholate Agar (Baltimore Biological Laboratories, Cockeysville, Maryland) for the selection of non-fastidious Gram-negative rods from clinical specimens. Nonlactose-fermenting colonies (as well as those whose lactose fermentation was obscured by a red pigment) were inoculated into a battery of test tubes including the DNase-Indole medium (2), Lysine Iron Agar (Baltimore Biological Laboratories), Ornithine-Motility medium (Decarboxylase Medium Base [Difco] with 0.5% 1-ornithine and 0.3% agar), and a modified Urea-Phenylalanine Medium (2). Results were read after 24 hr incubation at 37°C. An identification scheme of DNase-positive bacteria is given in Table I. If the organism proved to be nonfermentative, it was checked in a second set of tests including: OF Basal Media (Difco) with 1 % glucose, 1 % maltose, and 1 % xylose, Trypticase Nitrate Broth (Baltimore Biological Laboratories) (also for motility), susceptibility to colistin (10 μg Sensi-Disc [Baltimore Biological Laboratories]; Kirby-Bauer method), and oxidase (Taxo N disc [Baltimore Biological Laboratories]) (3). Further tests for confirmation of the presence of unusual strains were done according to the methods of Edwards and Ewing (4): for halophilic strains, 3 % NaCl was added to the test medium. Sensitivity tests were performed using the Kirby-Bauer technique.

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TABLE I

Identification of Typical DNase-positive Nonlactose Fermenters (or Red Pigmented Gram-negative Rods)—24 Hr. Readings

DNase	Indole	Ornithine	Motility	Lysine	H2S	Urea	Phenylalanine	Diagnosis
				(Lys. Iron Ag.)				
+	+	+	+	+		–	–	Vibrio[1]
+	+	-	+		–	–	$-/+^{w}$	Aeromonas hydrophia
+/–	+		+	-	–/+	+	+	Proteus vulgaris[2]
$+^{w}$	–	+	+	+	+	-	–	Arizona hinshawii
+	–	+	+	+		+	–	Serratia marcescens
+	–	+	+	+	–	–	–	S. marcescens, S. liquef[3]
+		+	+		–	–	–	S. liquefaciens
+	-		+	+/–	-	–	–	S. rubidaea
+	–	–	+	+		–/+	-	Pseudomonas maltophilia
+		+	+		+	–/+		P. putrefaciens
+	–		+	-	–	–	–	P. diminuta

+, positive; –, negative; /, or; w, weak.

[1] To differentiate between V. cholerae, V. parahaemolyticus, and V. alginolyticus, use Triple Sugar Iron Agar, Voges-Proskauer reaction, growth at 42°C, and growth with 0–10% NaCl (see text).

[2] Part of the strains are DNase-negative.

[3] To separate the two species, use arabinose fermentation.

Results and Clinical Findings

During the observation period, the following numbers of DNase-positive Gram-negative strains (one strain per patient per site) were isolated: S. marcescens and S. liquefaciens, 441; P. vulgaris, 55 (representing about one-half of the strains isolated), A. hydrophila, 25; P. maltophilia, 93; and P. putrefaciens (with H2S), 20.

The uncommon strains included: V. parahaemolyticus (from wounds), 3; *P. putrefaciens* (without H2S), 3; and *S. rubidaea*, 2.

Other DNase-positive species (V. alginolyticus, V. cholerae, Arizona hinshawii (2), P. diminuta (5)) were not isolated. For a listing of DNase reactions, we refer to Martin and Ewing (6).

V. parahaemolyticus. The characteristics of the three strains from wounds (other isolates were not observed) are listed in Table II. They are in line with typical strains of V. parahaemolyticus ("subgroup 1" of Sakazaki et al (7)). Strains # 7653 and 1206 grew in the normal laboratory media while strain # 5333 required the addition of 3 % NaCl for growth in certain media. All were resistant only to ampicillin and carbenicillin and required no salt addition to Mueller-Hinton Agar (Baltimore Biological Laboratories). Strain # 7653 was inadvertently discarded; the other two were sent out for serotyping and Kanagawa testing*.

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* I thank Dr. Morris Fishbein, Food Microbiology Branch, Food and Drug Administration, Washington, D.C. for performing serotyping and Kanagawa testing and for confirming our biochemical results.

TABLE II

Characteristics of Three Strains of V. parahaemolyticus

Test	Strain # 7653	Strain # 1206	Strain # 5333[2]
Oxidase	+	+	+
DNase	+	+	+
Lactose[1]	–	–	–
Indole	+	+	+
Ornithine	+	+	+
Motility	+	+	+
Lysine	+	+	+
H2S (TSI)	–	–	–
Urea	–	–	–
Phenylalanine	–	–	–
Arginine	–	–	–
Gelatin	+	+	+
TSI slant/butt	alkaline/acid	alkaline/acid	alkaline/acid
Voges-Proskauer	–	–	–
Growth at 42C	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Mannitol	+	+	+
Trehalose	+	+	+
Sucrose	–	–	–
Morphology	Pleomorphic Gram-negative rods on blood agar		
Kanagawa	not done	–	–
Serotype	not done	04 K?	untypable
Growth in trypticase with			
0% NaCl	–	–	–
3% NaCl	+	+	+
6% NaCl	+	+	+
8% NaCl	+	+	+
10% NaCl	–	–	–
Sensitive to	Kanamycin Nalidixic Acid Nitrofurantoin Gentamicin Tetracycline Colistin Cephalothin	Kanamycin Nalidixic Acid Nitrofurantoin Gentamicin Tetracycline Colistin Cephalothin	Kanamycin Nalidixic Acid Nitrofuratoin Gentamicin Tetracycline Colistin
Intermediate to	none	none	Cephalothin
Resistant to	Ampicillin Carbenicillin	Ampicillin Carbenicillin	Ampicillin Carbenicillin

[1] On Desoxycholate Agar; [2] required 3% NaCl addition for most media (see text).

Strain # 7653 was cultured from a crush injury wound of the distal phalanx of the right fifth finger which the patient, a 32-year-old male, had sustained through a cut with a hedge cutter. No therapy had been administered until the patient was seen two weeks after the accident. The wound culture grew many colonies of V. parahaemolyticus, P. putrefaciens (with H2S), enterococci, and anaerobic streptococci; and a moderate number of colonies of Escherichia coli and Klebsiella pneumoniae. The lesion healed on debridement and exposure of the distal phalanx with application of a flap.

Strain # 1206 was cultured from a draining chronic osteomyelitis of the left knee area of a 63-year-old male dating back to a hunting accident in 1938. Cultures taken in March, 1974 after dicloxacillin treatment, were negative for mycobacteria but grew many colonies of V. parahaemolyticus, P. morganii, and Staphylococcus aureus; one month later, additional growth of E. coli, anaerobic streptococci, and peptococci was observed. The patient's condition improved on oral gentamicin treatment; later, he was put on a trimethoprim-sulfamethoxazole regimen. No further cultures have been obtained so far.

Strain # 5333 was cultured in small amounts from a leg wound, together with many colonies of S. aureus. The patient, a 9-year-old girl, had fallen from a bicycle three weeks previously and had sustained a pretibial laceration which was debrided and sewed up on the same day. A superficial infection had developed at the time of admission which responded well to soaks. No antibiotics were administered, and no follow-up cultures were taken.

We could not elicit a history of contact with sea water from any of the patients.

P. putrefaciens. The characteristics of the three strains are listed in Table III. Basically identical in biochemistry and antibiograms, they belong to group 1 of Riley et al (8) in oxidizing maltose and failing to grow on SS Agar (Baltimore Biological Laboratories) and at 7% NaCl concentration. All failed to form H2S in Triple Sugar Iron Agar (Baltimore Biological Laboratories), Lysine Iron Agar, and with a lead acetate strip. They were sensitive to all antimicrobials tested. Further investigations elsewhere* showed that they also grew at 2 C, had a GC content of 49 %, and yielded a small number of apparent mutant colonies that formed H2S when tested on pour plates of peptone iron agar (9).

Strain # 9267 originated from the sputum of a 73-year-old man with chronic obstructive lung disease and arteriosclerotic heart disease. He was admitted in acute pulmonary edema. Sputum taken at that time contained many neutrophils and pseudomycelial yeast forms, Gram-positive and Gram-negative rods. Culture grew many colonies of S. aureus and a moderate number of colonies of K. pneumoniae, P. putrefaciens, Haemophilus sp., and yeast forms. The patient recovered under appropriate therapy without antibiotics. No follow-up cultures were taken.

Strain # 4321 was cultured from the ascitic fluid of a 55-year-old female who had been admitted for a papillary adenocarcinoma of the ovaries with extensive fibrous adhesions and metastases to the peritoneal cavity and the adjacent lymphodes. Irradiation had been applied to the abdomen. The culture, taken shortly before death, revealed a mixture of P. putrefaciens, E. coli, Enterobacter cloacae, K. pneumoniae, enterococci, Clostridium sp., Bacteroides fragilis, B. oralis, and yeast forms.

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* R. E. Levin: Characteristic of weak H2S-Producing Isolates of Pseudomonas putrefaciens from Human Infections, Antonie van Leeuwenhoek 41:569–574, 1975 (published after completion of this manuscript).

TABLE III

Main Characteristics of Three Strains of P. putrefaciens—24 Hr. Readings

Test	Strain # 9267	Strain # 4321	Strain # 290
Oxidase	+	+	+
DNase	+	+	+
Motility	+	+	+
Lysine	–	–	–
Ornithine	+	+	+
H2S (TSI, LIA)	–	–	–
Urease, Indole	–	–	–
Phenylalanine	–	–	–
TSI slant	alkaline	alkaline	alkaline
butt	no change	no change	no change
OF glucose	–	+[d]	–
OF maltose	+	+	+
OF xylose		–	–
Nitrate to nitrite	+	+	+
Pigment	tan	tan	tan
Growth on SS Agar	–	–	–
Growth (Trypticae)			
w/0% NaCl	+	+	+
w/7% NaCl	–	–	–
Lactose[1]	–	–	–
Morphology	Gram-negative rods on blood agar		
Sensitive to	Kanamycin Nalidixic Acid Nitrofurantoin Gentamicin Tetracycline Colistin Cephalothin Ampicillin Carbenicillin	same as # 9267	same as # 9267

[d] Delayed (more than 24 hr.). [1] on Desoxycholate Agar.

Strain # 290 originated from the sputum of a 61-year-old male who was on treatment with isoniazid, ethambutol, and rifampin for tuberculosis of both lungs. The culture, which had been taken routinely two months after his treatment had been started, grew a mixture of P. putrefaciens, K. pneumoniae, P. fluorescens, and A. hydrophila, with no signs of acute inflammation. There were no follow-up cultures.

S. rubidaea. As listed in Table IV, both strains were pigmented; their lactose-fermenting trait was thus not immediately evident on Desoxycholate Agar. They conformed to average strains of S. rubidaea (10); lysine decarboxylation was delayed beyond 48 hours. Both strains were resistant to cephalothin and tetracycline, one also to ampicillin and carbenicillin.

Strain # 1 was detected in a routine throat culture of a male child admitted to the Emergency Room for burns. Acinetobacter anitratus was also recovered from that culture.

Strain # 120 was cultured from the sputum of a 70-year-old male after

TABLE IV

Main Characteristics of Two Strains of S. rubidaea

Test	Strain # 1	Strain # 120
Oxidase	–	–
DNase	+	+
Indole	–	–
Motility	+	+
Ornithine	–	–
Lysine	– (+ after 72 hrs)	(+ after 72 hrs)
Lactose 1)	+	+
Voges-Proskauer	+	+
Gelatin	+	+
TSI slant	Acid	Acid
butt	Acid	Acid
H2S	–	–
Gas	+	+
Phenylalanine	–	–
Urea	–	–
Citrate (Koser)	+	+
Pigment	red (on Desoxycholate Agar)	red
Morphology	Gram-negative rods on blood agar	
Sensitive to	Nalidixic Acid	Nalidixic Acid
	Nitrofurantoin	Nitrofurantoin
	Gentamicin	Gentamicin
	Chloramphenicol	Chloramphenicol
	Colistin	Colistin
	Ampicillin	
	Carbenicillin	
Intermediate to	Kanamycin	none
Resistant to	Cephalothin	Cephalothin
	Tetracycline	Tetracycline
		Ampicillin
		Carbenicillin

endarteriectomy and sympathectomy for thrombosis of the right iliac artery. Postoperatively, he developed fever and bilateral basilar lung infiltrates while on carbenicillin. The sputum contained few white cells, and K. pneumoniae, Erwinia, and S. rubidaea in moderate amounts. The patient recovered without a change in therapy although the organisms were uniformly resistant to carbenicillin.

Discussion

The routine use of a DNase medium proved to be of substantial value in the 24-hr differentiation of several bacterial species (2): S. marcescens–S. liquefaciens from Enterobacter spp., A. hydrophila from indole-positive Erwinia and from decarboxylase-negative E. coli strains, and P. maltophilia from other oxidase-negative non-fermenters. Had the test not been used, the V. parahaemolyticus isolates most likely would have been identified as E. coli, and the non-H2S-producing P. putrefaciens as "Pseudomonas sp." Nonpigmented S.

rubidaea strains (10) (which we did not observe) could be mistaken for Erwinia.

V. parahaemolyticus. Tissue strains of this marine organism, which can be the causative agent of human gastroenteritis (7), have rarely been reported; we had earlier described one Kanagawa-negative strain from a wound which, like strain # 5333, did not grow in normal identification media (except DNase medium) without the addition of salt (11). The data of our strains point to intra-species variations in the NaCl requirement for growth which ranks from 0.5% (i.e., the concentration in most laboratory media) to 8.0% (7). On blood agar, halophilism is less marked (7); Mueller-Hinton Agar (concentration of NaCl ci. 1 % (11)) supported growth of all our strains.

Growth at 42°C and at 7% NaCl ruled out V. cholerae; the positive Voges-Proskauer reaction, the alkaline Triple Sugar Iron Agar slant, and the failure to grow at 10% NaCl differentiated our strains from V. alginolyticus.

Although these strains were obviously exogenous, no contact of the wounds with salt water could be established. The patients, however, all hailed from the East Coast of the United States. In view of the mixed cultures, the pathogenic role of our strains remains unclear but the negative Kanagawa reaction at least suggests a lack of enteropathogenicity (12). The antimicrobial sensitivity corresponds to that of our earlier isolate (11) and to that observed on 20 strains by Chatterjee et al (13); it is discussed in an earlier paper (11).

P. putrefaciens. This species has been isolated from human sources (5, 8, 14) although its pathogenicity has only rarely been proven beyond doubt (5). It is a free-living organism (5) that normally forms H2S in Triple Sugar Iron Agar or in Kligler's Iron Agar. Non-H2S-forming variants have been described by Levin (9) from spoiling fish but are considered rare by the author. In the absence of H2S formation, a tan pigment and susceptibility to many antimicrobials, even to cephalothin and nitrofurantoin, are suggestive of P. putrefaciens (5). Members of group 1 of Riley et al (8) and Levin (14) have a GC content of 47.8–50.8% (14) which falls within the range of Vibrio (40–50%) and is outside the range of Pseudomonas (57–70%) which encompasses the GC of members of group 2 (14)). Strain # 4321 must have originated in the intestine; the origin of the respiratory strains is unclear, as is the pathogenic potential of these isolates.

S. rubidaea. This species, first described as an epiphyte on plants (15), has only very rarely been described from clinical specimens (10, 16): in one series, only three of 85 Serratia strains belonged to the species S. rubidaea, and all came from the respiratory tract (16). For the differential diagnosis between S. marcescens and S. liquefaciens, the negative ornithine decarboxylase tests and the lactose fermentation are important (10). Both of our strains occurred in mixed culture and are of indeterminate pathogenicity. Strain #1 was certainly community-acquired while strain # 120 may have been acquired in the hospital. The relatively high antimicrobial susceptibility is unusual for S. marcescens and S. liquefaciens (17) but goes along with data from three other strains (16). Noteworthy is the fact that the carbenicillin-resistant strain # 120 was cultured after the patient had been on carbenicillin.

In conclusion, it can be expected that further reports on similar strains will be published since the DNase test should soon belong to the routine instrumentarium of the clinical bacteriologist.

Summary

Inclusion of a deoxyribonuclease-indole medium in the identification battery for non-lactose-fermenting Gram-negative rods led not only to a 24-hour identification of Serratia marcescens–S. liquefaciens, Proteus vulgaris, Aeromonas hydrophila, and Pseudomonas maltophilia, but also to the detection of three strains of Vibrio parahaemolyticus from wounds, three strains of non-H2S-forming Pseudomonas putrefaciens from sputum and peritoneal fluid, and two pigmented strains of Serratia rubidaea from the respiratory tract. Two strains of V. parahaemolyticus grew in all identification media, in spite of the halophilism of the species. The P. putrefaciens strains belonged to group 1 of Riley et al. and Levin, and showed characteristic antimicrobial sensitivity and pigmentation. The S. rubidaea strains were more sensitive than the average S. marcescens and S. liquefaciens strains. All isolates occurred in mixed culture and were of indeterminate pathogenicity.

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Isolation and Characterization of Yersinia Enterocolitica

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The Yersinia

In the 1957 edition of *Bergey's Manual* (1), the genus Pasteurella was described as consisting of 9 species. Until relatively recently only four of these were considered to be important in clinical medicine: P. pestis, P. pseudotuberculosis, P. multocida and P. tularensis. By 1961, in recognition of its fastidiousness and specific growth factor requirements, P. tularensis was removed from the pasteurellae and placed in the new genus Francisella. As early as 1944, van Loghem (2), using conventional taxonomic criteria, proposed that P. pestis and P. pseudotuberculosis be separated from the Pasteurella and placed into a separate genus he named Yersinia (in honor of the French bacteriologist, A. J. Yersin, who first isolated the plague organism). Using numerical taxonomy, Talbot and Sneath (3) and in 1965 Smith and Thal (4) demonstrated a clear division of pasteurellae into 2 groups, i.e., those oxidase-positive (containing cytochrome c in their respiratory electron transport chain), and those oxidase-negative; they also noted close relationships of the latter with some Enterobacteriaceae. Smith and Thal also showed that an organism designated "Pasteurella X," just then beginning to be isolated with increasing frequency from animal infections in various European countries, also clearly belonged with the oxidase-negative group. They then proposed that the oxidase-positive members of the genus be considered the true Pasteurella and that the oxidase-negative species (P. pestis, P. pseudotuberculosis, "Bacterium X") should be considered members of the genus Yersinia. This was accomplished in the 1974 eighth edition of *Bergey's Manual* (5), where Mollaret and Thal included these organisms in Yersinia and placed the genus in the family Enterobacteriaceae.

The reasons for placing the Yersinia in the family Enterobacteriaceae can be summarized as follows: (a) Both groups are fermenters, i.e., utilize carbohydrates by fermentative pathways; (b) both reduce nitrate to nitrite; (c) two of the three Yersinia, when motile (at 22–25 C but not at 37 C) have peritrichous flagella, like the Enterobacteriaceae; and (d) both groups are insensitive to bile salts, i.e., they grow on MacConkey agar (6, 7). In addition, antigenic relationships among the 2 groups exist (8), the DNA base composition of Yersinia is close to that of several Enterobacteriaceae (9), and DNA/DNA homology studies support the relative closeness of the 2 groups (10).

The 3 species of Yersinia are primarily found in animals (zoonotic bacteria):

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Y. pestis in rodents and insect vectors, and the other 2 species in a wide variety of mammals, and birds, where they may cause extensive epizootic outbreaks or may persist in healthy carriers.

In man, Y. pestis is the causative agent of plague, while Y. pseudotuberculosis and Y. enterocolitica are responsible for a variety of syndromes, the most common clinical infections being enterocolitis, and acute mesenteric lymphadenitis, often mimicking acute appendicitis (6, 11).

Yersinia are relatively large (0.5–1.0 by 1–2 μm) coccobacillary, ovoid- or rod-shaped Gram-negative bacteria, nonmotile at 37 C, but Y. pseudotuberculosis and Y. enterocolitica are motile at 22–25 C. The organisms are facultative anaerobes (grow aerobically or anaerobically), with good growth on ordinary media, including MacConkey agar; Y. enterocolitica produces very small translucent colonies on SS agar after 18 hr. All Yersinia ferment glucose, maltose, mannitol, trehalose, glycerol, xylose and fructose with acid but no gas; they usually do not ferment lactose but produce β-D-galactosidase. They do not ferment dulcitol, inositol, raffinose and melezitose. On initial isolation, Yersinia (especially Y. enterocolitica) strongly resemble certain other Enterobacteriaceae (especially anaerogenic "atypical coliforms," Proteus morganii, P. rettgeri, shigellae, Providencia) and are easily misdiagnosed (12, 13, 14).

With the combined use of triple sugar iron (TSI) agar and lysine iron (LIA) agar slants (13, 15), Yersinia can be presumptively differentiated from a large number of other fermentative Gram-negative bacilli, with further tests to be undertaken for definitive identification. In TSI the reactions are: Alkaline slant/acid butt, no gas (Y. pestis, Y. pseudotuberculosis); acid slant/acid butt, no gas (Y. enterocolitica, due to fermentation of sucrose; occasional Y. pseudotuberculosis). LIA reactions are: Alkaline slant/acid butt.

Any lactose-negative Gram-negative rod which does not produce H_2S in TSI, is oxidase negative, motile only at room temperature, and is urease-positive on Christensen urease agar in 3–24 hrs. but is phenylalanine-negative, should be suspected of being Y. enterocolitica or Y. pseudotuberculosis, and its further identification pursued.

Yersinia enterocolitica

The designation Y. enterocolitica was proposed by Frederiksen in 1964 (16) for an organism described variously as Bacterium enterocoliticum, Pasteurella pseudotuberculosis type B, and Pasteurella X. Y. enterocolitica is morphologically and culturally related to Y. pseudotuberculosis, but differs from it with regard to serology, pathogenicity in animals, bacteriophage sensitivity, and certain biochemical reactions. It was first isolated (as Bacterium enterocoliticum) in 1933–43, in New York State (17) from 2 patients with cervical adenitis and three with acute enteritis and terminal ileitis; the isolates, of which two are extant, have been identified in retrospect as Y. enterocolitica. Six additional cases in the U. S. were reported from 1940–47; no other cases of human infection were recognized in the U. S. for the next 21 years when a case of Y. enterocolitica meningitis with panophthalmitis was reported from Missouri (6). In contrast, over 2,000 cases in humans were diagnosed since 1964 in Europe. By 1974, some 60 culture-proven cases were known to have occurred in the U. S. and 256 cases, mostly enteritis, were reported in Canada (18). Human Y. enterocolitica infections have also been reported in Central and South Africa, Russia and Japan.

Clinical infections. In addition to the most common forms of Y. enterocolitica infection, i.e., enterocolitis and acute mesenteric lymphadenitis, other manifestations include erythema nodosum, typhoidal syndromes, nonsuppurative arthritis (19), and generalized septicemia. The organism has also been isolated in meningitis (6), abscesses of the spleen, colon and neck, local infections at indwelling catheter site, cholecystitis, eye infections, urine, wounds (11), and purulent arthritis (20). Interfamilial outbreaks (21), hospital-acquired infections (22), and institutional outbreaks (23, 24) occur.

Isolation. Y. enterocolitica has been isolated in man from blood, effusions from serous cavities, mesenteric lymph nodes, wounds, abscesses, feces, spinal fluid, and organ specimens. Generally, isolation and recognition of the organism from areas usually sterile is not difficult, since it does grow well on ordinary media (blood agar, MacConkey agar, etc.). Isolation from feces, however, requires additional technics and attention.

Y. enterocolitica grows slower at 37°C than other enteric organisms; on SS agar its colonies are minute in size after overnight incubation at 35–37°C, but after 48 hr the colonies are smooth and colorless, resembling those of Shigella. Although selenite (25) and Rappaport (26) broths have been recommended for enrichment culture, it seems much preferable to rely on the use of SS and MacConkey agar plates, incubated at 22–28°C (room temperature). This is accomplished by some workers by using duplicate sets of plates, one set incubated at 35–37°C and the other at room temperature. Toma (27) and Wauters (28) both agree that the use of bile salts media (pH 7.2–7.4) with low temperature incubation *and* the use of a stereomicroscope with oblique transillumination of plates for examination of colonies is the most convenient method with the highest yield of isolation. Jacobs (19) describes the use of two MacConkey plates with incubation of one at room temperature, a practice that can easily be incorporated into present laboratory routines. Inoculation of stools in phosphate buffer solution (pH 7.6) kept for 10 days at 8°C before subculture to SS agar, yields more isolations than direct culture on SS agar (24), and refrigeration in isotonic saline, with or without 25 μg/ml potassium tellurite has been shown to result in selective enrichment (14, 29). Whenever the organism occurs in epidemic form, it can be recovered from the stool in a high percentage of cases, assuming a strong index of suspicion both to grow and recognize it (20).

Y. enterocolitica has been isolated in Europe from both healthy and sick swine, dogs, rabbits, chinchillas, cows, sheep, and horses; from deer, chinchilla and dogs in the United States; and, in addition to the above, from cats, beavers, raccoons, various birds and oysters in Canada (18).

The organism has been recovered from water specimens (well water, streams) in Europe as well as North America (20, 27).

Recognition and identification. On blood agar, after overnight incubation, the lactose-negative colonies are round and unremarkable, resembling those of other enteric organisms. On MacConkey agar they are 1 to 2.5 mm in size, and on SS agar they are tiny, with a hazy rim, after overnight incubation. SS and MacConkey plates of stool (or other mixed) cultures should be examined with a dissecting or plate microscope (13× magnification), using oblique illumination with the light (100-watt bulb) reflected at an angle of approximately 45° from the concave side of the mirror. When examined in this manner, the very small colonies (<1 mm) are grayish, translucent with a fine hazy outer edge (27). Such colonies should be picked with a straight platinum needle and inoculated into appropriate differential media (TSI, etc.). For mixed cultures, the use of SS agar (pH 7.2–7.4) results in the highest number of Yersinia isolations, with MacConkey agar ranking next; XLD and Hektoen media are not suitable for recognition since the organisms ferment sucrose and salicin in Hektoen agar and sucrose and xylose in XLD agar (27).

On Gram staining, the Gram-negative organism is generally coccobacillary, oval or rod shaped; little or no bipolarity is seen even when stained with Wayson's stain.

On TSI, Y. enterocolitica reactions are those of an anaerogenic E. coli (27): both the slant and the butt are acid (due to sucrose fermentation) without gas formation, and no H_2S is produced. Urea agar slants typically turn positive in 3–24 hrs. but phenylalanine is negative.

Differential characteristics of Yersinia enterocolitica and the 2 other Yersinia species are shown in Table I. See Table II for typical biochemical characteristics of Y. enterocolitica (but see discussion of biotypes and atypicals, below).

The following salient points should be noted:

1. *Motility.* The organism is motile at 25°C but not at 35–37°C. Therefore, it is advisable to perform motility tests at both temperatures.

2. *Differentiation of Y. enterocolitica (and Y. pseudotuberculosis) from Salmonella:* This is easily accomplished because Salmonella are (a) motile at 37°C; (b) urease negative; (c) most types produce gas; (d) utilize citrate; (e) most are H_2S positive and (f) lysine positive.

3. *Differentiation of Shigella:* Species of Shigella are (a) urease negative, (b) nonmotile at 37°C *and* at 25°C.

4. *Differentiation of Proteus:* Species of Proteus are (a) β-D-galactosidase negative, (b) motile at both room temperature and 37°C and (c) are usually phenylalanine deaminase positive.

5. *Indole:* The majority of United States isolates are indole positive, in contrast to those in Europe and in Canada which are indole negative (or indole late, i.e., serotype 0:9, the second most prevalent human serotype in Europe).

6. *Temperature dependence:* In addition to motility, the tests for β-D-galactosidase (ONPG), maltose fermentation, and acetoin production (Voges-Proskauer) show a marked temperature dependence. These tests are mainly positive at room temperature and delayed or negative at 37°C (7).

For definitive identification, those tests in italics (Table I) should be

TABLE I

Differential Characteristics of the Genus Yersinia

	Y. pestis			Y. pseudotuberculosis			Y. enterocolitica		
	Sign	%+	(%+)*	Sign	%+	(%+)*	Sign	%+	(%+)*
Motility 25°C		0		(+)or+	40	(45)	+	96	
37°C	−	0		−	0			0	
Urease		0		+	100		+	90.7	(6.7)
Phenylalanine deaminase	−	0		−	0			0	
Catalase	+			+			+		
Oxidase					0		−	0	
Citrate (Simmons)					0			0	
H_2S (TSI)	−				0			0	
Lysine decarboxylase	−			−	0			0	
Arginine dihydrolase	−				0		−	0	
Ornithine decarboxylase					0		+	90.7	(6.7)
Esculin hydrolysis	+	100		+	100		d	14.7	(21.3)
β-galactosidase 37°C	+			+or	70		−or+	24.0	
25°C				+or	80		+	93.3	
Nitrate reduction	+			+	95.0		+	98.7	
Indol		0			0		+or−**		
Methyl red 37°C	+			+^{w}	100		+	100.0	
25°C				+	100		+	100.0	
Voges-Proskauer 25°C					0		+or	86.7	
37°C				−	0			0	
Fermentation of:									
Glucose, acid	+			+	100		+	100	
Lactose 37°C				−	0			0	(5.3)
25°C								0	(2.6)
Sucrose					0		+	96.0	
Adonitol				+	0		−	0	
Rhamnose		0			100		−	1.3	
Salicin	or +	19.0		(+)	5	(95)	d	13.3	(14.6)
Sorbitol	−or+				0		+	98.7	
Cellobiose					0		+or(+)	88	(12.0)
Xylose				+	100		d	36.0	(2.7)
Mannitol	+			+	100		+	100	
Maltose 37°C	+			+	90		d	42.7	(50.6)
25°C				+	100		+	98.7	(1.3)
Raffinose				−or+	20		−	6.7	
Arabinose	+			+or(+)	50	(45)	+	98.7	(1.3)
Melibiose	d			+	90.0	(10.0)		0	
Trehalose	+			+	100		+or(+)	89.3	(9.3)

Percentages from: Darland, G., Ewing, W. H. and Davis, B. R., 1975, *The Biochemical Characteristics of Yersinia enterocolitica and Yersinia pseudotuberculosis*, DHEW Publ. No. (CDC) 75-8294, Center for Disease Control, Atlanta, Ga.

* Delayed reactions, 3 days or more.

** Majority of strains isolated in the U. S. have been indole-positive; majority of those isolated in Europe have been indole-negative.

Key (refers to reactions with percentages given): +, 90% or more positive within 1 or 2 days; (+), positive reaction after 3 or more days (decarboxylase tests: 3 or 4 days); −, no reaction (90% or more); +or−, most cultures positive, some strains negative; −or+, most strains negative, some cultures positive; +or(+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions: +, (+), or −

performed, followed by the additional tests listed. A number of the recently introduced micromethods and multitest systems for the identification of Enterobacteriaceae (API, RB, Pathotec, Enterotube) have incorporated Y. enterocolitica in their computer-based identification schemas, but at this time there is not sufficient experience with fresh clinical isolates for judging their performance in this respect. Isolates resembling Y. enterocolitica should be submitted to state public health laboratories for verification or confirmation of identification. Serotyping (see below) of confirmed isolates and antibody titer measurements are done at the Plague Research Center, Fort Collins, Colorado.

Biotypes and "atypical" isolates. Strains described and collected in the 1960's under the collective designation Y. enterocolitica are biochemically heterogeneous and differ in a number of reactions. Wauters (31) reinvestigated these strains and divided the entire group into 5 biotypes, characteristics of which are given in Table III. Knapp and Thal (32) disagree with Wauters' biotype classification and describe four groups according to their production of indole, acid in xylose, esculin and salicin. Their group 1 consists of strains with the typical biochemical reactions of Y. enterocolitica (Tables I and II) the second group includes indole and xylose positive strains. Group 3 strains are indole, xylose, esculin and salicin positive, some also producing gas in glucose, while group 4 consists of a few strains, some of which are lactose positive. In their opinion, the strains of group 3 and 4 do not belong to the species Y. enterocolitica.

Recently, Bottone and coworkers (33) reported the isolation, from non-mesenteric sources, of 12 atypical strains of Y. enterocolitica, which differed from other Y. enterocolitica previously described in the United States and in Europe in that they fermented rhamnose and raffinose (at 22°C rapidly), and salicin; they also utilized (Simmons) citrate, and failed to grow on SS and Hektoen agars. The 12 strains belong to serotype 17. These strains cannot be placed with certainty in any of the biotypes described above.

TABLE II

Typical Biochemical Characteristics of Y. enterocolitica

Motility 25°C	+	Rhamnose	–
37°C	–	Sucrose	+
		Salicin	–
Urease	+	Glucose (gas)	–
Phenylalanine deaminase	–	Lysine decarboxylase	–
β-D-galactosidase 25°C	+	Arginine dihydrolase	–
Citrate	–	Ornithine decarboxylase	+

Note: All Yersinia are oxidase-negative.

TABLE III

Biotypes of Yersinia enterocolitica (30)

Characteristics	Biotypes				
	1	2	3	4	5
Lecithinase	+	–	–	–	–
Indol	+	(+) 29°C	–	–	–
Xylose, 48 hrs	+	+	+	–	–
Nitrate reduction	+	+	+	+	–
Trehalose	+	+	+	+	–
Ornithine decarboxylase	+	+	+	+	–
β-galactosidase	+	+	+	+	–
Lactose oxidation (OF medium), 48 hrs	+	+	+	–	–

Interestingly, a number of strains isolated from water in Canada (18, 27) are also biochemically atypical: Some are rhamnose positive and at least one (27) is serotype 17.

Serology. Based on thermostable O antigens, Winblad (34) established an antigenic schema with 9 serogroups; this was followed by Wauters' schema of 17 serogroups (31). Recently, Knapp and Thal (32) proposed a simplified schema containing 6 O-groups. It is expected that further O-groups and subgroups will be proposed in the future. Agglutination or hemagglutination tests are used for antigenic analysis. Most isolates from patients in the United States are serotype 8 (not seen in Europe), most Canadian strains are type 3, and the most frequent European isolates are types 3 and 9. About 2.5% of normal individuals (in Sweden) possess significant Y. enterocolitica antibody titers. In frank infections antibody titers are demonstrable by agglutination or hemagglutination; usually absent at the onset, they rise rapidly within the first few days of illness, with maximal titers obtained within 1–2 weeks, and usually drop significantly within 1–2 months. Serological diagnosis is reliable only if performed by reference laboratories with a good collection of serotypes. Marked cross-agglutination reactions have been observed between Brucella abortus and Y. enterocolitica serotype 9.

Animal pathogenicity. Until recently, Y. enterocolitica was described as non-pathogenic in the usual laboratory animal (guinea pig, mice, white rabbits) by the intraperitoneal, intravenous or subcutaneous routes (unlike Y. pseudotuberculosis which is pathogenic in all these animals). However, in 1973, Carter et al (35) described a clinical isolate highly virulent for mice by the IV and oral routes; similarly, in 1974 Quan et al (36) reported killing of mice by two human isolates of Y. enterocolitica and one isolate of flea origin. An additional seven of their strains produced pathologic lesions in mice after subcutaneous inoculation.

Bacteriophage. Nicolle et al (37), at the Pasteur Institute (Paris) isolated strains of Y. enterocolitica phages which allow grouping of Y. enterocolitica strains into 9 main phage types; type 10 consists of strains not typable by phage.

Antimicrobial susceptibility. The organism is reported by many investigators to be susceptible in vivo to kanamycin, colistin, chloramphenicol, streptomycin and, recently, to gentamicin, and the combination trimethoprimsulfamethoxazole. Resistance to cephalothin and variable resistance to ampicillin and neomycin have been noted. Recommended treatment of systemic Y. enterocolitica is not yet established; chloramphenicol and colistin have been used successfully in several cases.

Coda

A rapid increase in the incidence of Y. enterocolitica infection in both man and animals occurred in Europe and Asia in the late 1960's. A similar increase, albeit a few years later, has taken place in Canada and the United States: Whether the latter is due to increased awareness and laboratory skill or represents an actual spread in man and animals is not known. The recognition of Y. enterocolitica as a pathogen in man, the increase in the variety of clinical forms recorded, and in the number of animal species infected raises a number of fundamental questions:

(a) Is this organism an example of the recent adaptation of a microbe to certain host species in which it has found a favorable biologic terrain (30)? There is some likelihood that this may be so, since there are two broad categories of Y. enterocolitica strains known, one of which was recovered from subjects with acute and sometimes fatal disease, while the second is comprised of strains from humans with minor infectious manifestations, or occasionally from healthy humans, or from clinically healthy animals. These latter strains may still be in search of a host (31).

(b) Are the classification, nomenclature, and identification procedures of Y. enterocolitica adequate and complete? The answer is clearly no, since the very large number of strains collected in the last decade is clearly biochemically heterogenous. The work of Bottone et al (33) uncovering a number of atypical strains, the finding of different atypical strains in widely dispersed water sources, and the obvious proliferation of "biotypes" point toward the possibility that, in addition to "typical" Y. enterocolitica, there are many other Yersinia strains that conceivably may form entirely new species. In fact, Winblad (personal communication) has tentatively grouped a large number of isolates into four additional proposal species.

(c) What is the source of human infection? The answer is not known. While it is reasonable to suppose that the portal of entry is the gastrointestinal tract, no single source has yet been unequivocally identified. Person-to-person transmission is one possibility (note incidence of interfamilial outbreaks), but foodstuffs and water are likely more important. In Europe, pigs and human beings share serotype 3 (biotype 4, phage type 8), and thus the pig is thought to be the reservoir for human infection (20). In one instance, at least, stream water (most likely contaminated by animal droppings) from which the organism with identical bio-, sero- and phage-type was isolated, seems to be almost certainly the source of infection (20). Certain serologic groups of Y. enterocolitica (i.e., 9 and 15) have so far been encountered in man only: There is no known animal reservoir for serotype 9 strains, and it may be that this type may be specific to man (30).

(d) What is the pathogenesis of Y. enterocolitica infections? Presently, little is known about it, however, much progress is expected in this area since the recent finding (discussed above) of strains with marked virulence for laboratory animals. Recently Carter (38) studied an experimental animal model infected with Y. enterocolitica and demonstrated exceptional pathological similarity between the naturally acquired infection in man and that induced in mice with the same strain of Y. enterocolitica. Such work promises much in the development of further diagnostic tests, therapeutic regimens, understanding of pathogenesis, and the host's immune response.

The increasingly large number of culture-proven human Y. enterocolitica infections makes it seem certain that the organism, and the disease, is far more prevalent than previously recognized. Further advances in the understanding of the organism and the host's response will depend on proper laboratory diagnosis and awareness of microbiologists, internists, surgeons, and pathologists of the possible involvement of Y. enterocolitica in a number of syndromes, especially in cases of unexplained diarrhea (i.e., inability to recover Salmonella, Shigella or enteropathogenic E. coli) and acute abdominal pain. Careful bacteriologic studies, accompanied by a strong index of suspicion and utilizing proper media and incubation temperature need to be performed for the isolation and recognition of Y. enterocolitica. Biochemical, antigenic and phage-type characteristics of strains isolated from humans will aid in epidemiologic investigations, directed toward solving the riddle of the reservoir(s) and mode(s) of transmission of the organism.

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The Ecology of the Genus Neisseria

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Introduction

The prokaryotic bacteria of the genus Neisseria colonizing man consist of some six defined species of Gram-negative, oval cocci which characteristically occur in pairs with the long axes parallel to the line of division (diplococci). Usually aerobic, but occasionally facultatively anaerobic, they are neither motile nor sporogenous but are distinguished by the production of catalase and the presence of oxidase, their colonies' turning purple upon application of tetra-methyl-p-phenylenediamine. The genus belongs to the family Neisseriaceae which has been extensively reorganized with the publication of the 8th Ed. of *Bergey's Manual* in 1974 (1). Their anaerobic analogues are now classified in a separate family, the Veillonellaceae, while the closely related Mimeae family of Gram-negative coccobacilli, the so-called "pseudogonorrhea" of the genera Mima and Herrellea, are now considered within the Neisserial family (Table I).

The genus takes its name from the German physician, Albert Ludwig Siegmund Neisser, who, in 1879, described diplococci in smears of purulent secretions from patients with acute gonorrhea and conjunctivitis of the newborn. The name "gonorrhea" itself is attributed to none other than Galen, in about 130 A.D. The causative organism of "cerebrospinal fever" was described some eight years after Neisser's report by his Austrian contemporary, Anton Weichselbaum, and was subsequently assigned to the genus Neisseria. These two organisms known in the Latin by their binomials, Neisseria gonorrhoeae and N. meningitidis, respectively, and in the vernacular as the gonococcus and meningococcus are together responsible for almost all human disease produced by the genus. The remaining members of the genus are almost invariably commensal with or accidental to the upper respiratory tract of man. The rarity with which they produce the common neisserial syndromes makes them of primary interest to the clinical bacteriologist who must distinguish them from the more pathogenic N. meningitidis and N. gonorrheae.

The Neisserial Syndromes

Diseases of Mucous Membranes

The Neisseriae are organisms of the moist mucous membranes of man and other animals. With the exception of the gonococcus they are rarely found far

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TABLE I

Classification of Gram-Negative Cocci and Coccobacilli

Old (1)		New (2)		DNA homologies (3)
Family	Genus and species	Family	Genus and species	
Neisseriaceae	Neisseria gonorrhoeae	Neisseriaceae	N. gonorrhoeae	Group I
	meningitidis		meningitidis	I
	sicca		sicca	Group II
	flavescens		flavescens	II
	hemolysans		mucosa	II
	subflava		subflava	II
	perflava		subflava	II
	flava		subflava	II
	catarrhalis		Branhamella catarrhalis	Group III
	caviae		B. caviae	III
	Veillonella sp.	Veillonellaceae	Veillonella sp.	
Mimeae	Oxidase negative Gram negative coccobacilli of genera Mima and Herellea	Neisseriaceae	Acinetobacter calcoaceticus	
	Mima polymorpha var. oxidans		Moraxella urethralis	
Brucellaceae	Oxidase positive Gram-negative coccobacilli of genus Moraxella		Moraxella sp.	

from the upper respiratory tract. Both morphologic and biochemical similarity between members of the genus as well as the presence of antigenically similar cell wall proteins and lipopolysaccharides (4) suggest that all Gram-negative cocci evolved from a common ancestor. Studies of DNA homology confirm this view, and, in addition to providing a basis for dividing the genus into three subgroups (Table I), confirm the close relationship between its two pathogenic members, N. gonorrhoeae and N. meningitidis (3). The evolutionary forces which disposed the gonococcus to leave its ancestral home on the respiratory mucosa in favor of that of the genital tract and the biochemical modifications this entailed are among the most intriguing and unanswered questions in Neisseriology. Nor is the split complete. Not only are pharyngeal gonococcal infections being increasingly recognized (5) but occasional instances of acute cervical and urethral "gonorrhea" have been ascribed to both the meningococcus (6) and, more rarely, other members of the Neisseriaceae.

In their chosen ecological niches the Neisseriae usually produce no detectable disease. From 3–30% of normal individuals harbor meningococci on their nasopharynx (carrier state) at any given time yet overt meningococcal disease rarely supervenes. Indeed, even during epidemics of meningococcal meningitis in Army recruits, when pharyngeal carriage of the causative organism may reach 95%, the incidence of overt systemic disease is less than 1.0% (7). Likewise, around 3% of all women harbor gonococci on their genital mucosa, some for many years, yet the annual incidence of overt systemic disease in women so colonized is also less than 1.0%. Since Homo sapiens is the only natural host of both Neisseriae, it seems reasonable to assume that the mucosae of the genital tract of women and of the upper respiratory tract of both sexes represent the natural reservoirs of these organisms with travel arranged by exhaled droplets on the one hand and sexual intercourse on the other. A high order of virulence for man, therefore, would not subserve their evolutionary purposes.

The low incidence of overt disseminated disease following colonization also suggests that host rather than parasite factors largely determine its development. The nature of these host factors has been identified for the meningococcus as complement-mediated bacteriolysis by circulating antibodies of the IgG and IgM classes (9, 10). These bactericidal antibodies act as a circulatory barrier to systemic dissemination of meningococci which might invade from the upper respiratory mucosa. Analogous host factors have not been identified for the gonococcus, but since the male urethra is the presumed inter-reservoir vector and since males consorting with infected females have a high risk (22%) of overt local infection but rarely develop systemic disease (11), it is reasonable to assume that important host defenses operate locally within the male urethra.

One plausible explanation not only for the increased incidence of local symptomatic infection of the male urethra as compared to the female genital mucosa with the gonococcus, but also for the evolutionary dissociation between the gonococcus and the meningococcus is that substances present in the male urethra during intercourse act as substrates for which the gonococcus has evolved enzyme systems to serve as catalysts for the elaboration of surface factors conferring either increased virulence and/or resistance to host defenses. In support of this view, Watt, Glynn and Ward (12) have demonstrated that gonococci grown on solid media containing prostate extracts are resistant to bactericidal antibodies, while these same strains cultured on routine laboratory media are quite sensitive. Richardson and Sadoff, of our laboratory, have demonstrated that gonococci, after passage through hamster kidney cells, elaborate a new surface antigenic marker (13), while other workers have demonstrated reversion of avirulent gonococci to a virulent phase after similar tissue culture passage (14).

Symptomatic mucosal infections appear to be most frequent on those sites which are normally sterile such as the male urethra and the conjunctiva, while the oropharynx and uterine cervix which are continually bathed in secretions containing larger numbers of various bacterial species are rarely involved with symptomatic inflammation following colonization by Neisseriae. Indeed, symptoms of gonococcal pharyngitis correlate better with the mechanical trauma of fellatio than with the presence of gonococci (5). Vaginitis, uterine cervicitis, pharyngitis, proctitis, which may mimic ulcerative colitis, and even pneumonia have been ascribed to members of the genus. When appropriately treated these infections are benign. Untreated mucosal infections, however, may lead to local complications such as urethral stricture formation or ulcerative keratitis with perforation; local spread such as salpingitis; or systemic dissemination and secondary localization, such as meningitis or endocarditis.

Syndromes of Dissemination

As has been noted, about 0.5% to 1% of individuals who harbor meningococci or gonococci on their various mucous membranes develop symptoms of dissemination or septicemia. Three distinct, but overlapping syndromes, have been identified. The first, marked primarily by the development of disseminated intravascular coagulation, occurs with meningococcal dissemination, but rarely with gonococcal. Known by such names as fulminant meningococcemia, Waterhouse-Friderichsen syndrome and purpura fulminans, it presents with widespread and often confluent purpura, circulatory collapse and death. The organism can be recovered from the blood as well as the purpuric skin lesions. The second, rather common with gonococcal dissemination but rare with meningococcal, is marked primarily by skin lesions which begin as a tender red papule and progress to a pustule with eschar formation. The gonococci can be seen on Gram stain, but rarely cultured, though the blood culture is positive in about 50%. An identical syndrome may rarely be produced by Moraxella urethralis (formerly called Mima polymorpha var. oxidans) as well as other Neisseriaceae. The third syndrome is a sterile arthritis or tenosynovitis which not infrequently occurs coincidental with the pustular dermatitis syndrome. Joint fluid is sterile as is the blood culture in the absence of coincidental pustular dermatitis. Its similarity to serum sickness and the failure to culture organisms from blood, skin or joint fluid have led some to speculate that it is an immune complex or hypersensitivity disease (15). These latter two syndromes are often combined and referred to as the "arthritis—dermatitis syndrome" (8). It is rare in males, but not uncommon in females. When due to the meningococcus it is referred to as chronic meningococcemia.

Syndromes of Secondary Localization

Following dissemination or contiguous spread the Neisseriae may produce localized purulent disease. Again, there is a dissociation between sites of disease, the meningococcus producing primarily meningitis, the gonococcus producing pyoarthrosis and pelvic abcesses. The pathogenic mechanism of gonococcal peri-hepatitis is unclear. The absence of pus has led some to attribute it to a hypersensitivity state, but it may also represent intraperitoneal spread. Endocarditis due to either organism occurs rarely but usually catastrophically.

Bacteriology

Obtaining Specimens

The bacteriology of the Neisseriae rightly begins with the obtaining of adequate specimens. From the above it should be clear that the specimens obtained will be dictated by the syndrome studied. For a patient with meningitis, cultures of cerebrospinal fluid and blood with standard media are adequate. For a patient with proctitis, however, a simple culture will reveal many coliforms but no Neisseriae, unless the coliforms are suppressed. Such suppression is accomplished by incorporating antibiotics into the initial culture media. Collectively, these inhibitory media are known as Thayer-Martin, though the antibiotics initially used by those workers have been supplanted. Currently a combination of Vancomycin, Colistin and Nystatin is usually employed (1). For throat cultures, a combination of Lincomycin and Polymyxin B is adequate (16). Specimens from the anus, vagina, uterine cervix, urethra or throat are routinely placed on Thayer-Martin media to suppress non-Neisserial flora, but it is not only unnecessary but contraindicated to use these selective media for culturing bodily fluids such as synovial, spinal, peritoneal or blood, since even at the permissive concentrations of antibiotics used, a small percentage of Neisseriae are inhibited (17). Identification of the Gram-negative diplococci in fluids other than blood may be facilitated by first centrifuging the specimen to concentrate bacteria in the pellet prior to plating on appropriate media. Other Neisserial genera are totally inhibited by the antibiotics used, which facilitates the separation of N. gonorrheae from the ubiquitous Moraxellae and Acinetobacteriae in cultures of the uterine cervix.

The Neisseriae are sensitive to drying, succumbing in a few hours, and are susceptible to various bacterial products such as bacteriocins and lipids. For this reason swabs of mucous membranes should be inoculated directly or be placed in an appropriate transport medium for transporting to the laboratory (18). Petechial and purpuric lesions are cultured by wiping the surface with alcohol, which must dry before pricking the lesion with scalpel point or needle, and expressing a drop of blood which is then streaked on chocolate agar. A portion of the first drop is also placed on a glass slide and stained by Gram's technique.

Cultural Characteristics

N. meningitidis and N. gonorrheae are metabolically rather demanding. They grow poorly on standard blood agar plates. Heated blood agar (chocolate agar) with or without antibiotics, supports growth nicely and should be routinely used. Mueller-Hinton agar may be substituted, but gonococci grow poorly unless enriched with a supplement such as isovitalex. Growth is augmented by incubation in a 10% carbon dioxide atmosphere at 35–37°C. Either a CO_2 incubator or a candle extinction jar will suffice. The optimum pH range is 7.2–7.6 and a moist atmosphere is essential.

After 18–24 hours incubation, plates are examined for typical colonies. Meningococci produce smooth, moist, elevated colonies which are grayish in color. The gonococcal colonies are similar but slower to develop, requiring 48 hours to reach an equivalent size. They are grayish-white in color. There is no hemolysis. After further incubation they increase in size and four distinct colonial types may be distinguished on the basis of size, discreteness and crenations of border, granularity, light transmission and degree of roughness. These colonial morphologies, denominated T1, T2, T3 or T4, are reversible phenotypic expressions which can be somewhat stabilized by repeated passage of single, typical colonies. T1 and T2 organisms possess classical pili (the functional unit by which microorganisms exchange genetic material), while T3 and T4 organisms do not. There is a broad correlation between "T type" and ability to produce exudative disease in the male urethra: T1 and T2 produce exudation readily; T3 and T4 poorly or not at all (19). Similar colonial variants are not observed with the meningococcus.

Identification

Confirmation of the presence of a Neisserial species is obtained by demonstrating the presence of indophenol oxidase. The culture plates are sprayed or flooded with a sterile 1% solution of tetramethyl-p-phenylenediamine. Oxidase-positive colonies turn a bright purple and may be immediately subcultured onto "sugar plates." Moraxella urethralis (Mima polymorpha var. oxidans) may be mistaken for a Neisseriae, since it also synthetizes oxidase, but is usually inhibited by the antibiotics in the initial culture media, as are the "saprophytic" Neisseriae.

Members of the genus Neisseria are differentiated by their ability to oxidize simple sugars (Table II), and by pigments produced by their colonies. Glucose, maltose, fructose and sucrose are added to an enriched medium such as cystine trypticase containing a litmus marker such as phenol red. Upon oxidation of the carbohydrate present, acid is produced which changes the color of the marker from red to yellow. It will be seen in Table II that, as luck would have it, the *g*onococcus oxidizes only *g*lucose and is the only Neisseriae to do so. The *m*eningococcus oxidizes *m*altose in addition to glucose. Members of the Moraxella and Acinetobacter genera oxidize none of the carbohydrates. Neisseria subflava which oxidizes both glucose and maltose can be distinguished from N. meningitidis by its yellowish-gray color and its ability to grow at 22°C. Occasional strains of both N. gonorrhoeae and N. meningitidis oxidize carbohydrates slowly. Sulfonamide resistant strains of N. meningitidis, in particular, may utilize maltose poorly. The addition of rabbit or human serum to the medium and the use of a heavy bacterial inoculum facilitates the test.

For a more detailed review of the identification and classification of the Neisseriaceae see references 2 and 20.

Final identification of N. meningitidis and N. gonorrhoeae is by serological means: the meningococcus being identified by agglutination based on its

TABLE II

Oxidation reactions of Neisseriae and Branhamellae

Species	Glucose	Maltose	Fructose	Sucrose
N. gonorrhoeae	+		–	-
N. meningitidis	+	+	–	-
N. sicca	+	+	+	+
N. mucosa	+	+	+	+
N. flavescens	–	–	–	
N. subflava	+	+	V	V
B. catarrhalis	–		–	–
B. caviae	-	–	-	

V = variable.

capsular antigen and the gonococcus by fluorescent antibody staining. Both methods will be discussed below.

Antibiotic sensitivity of Neisseriae

Two major clinical problems have developed due to increasing resistance of Neisseriae to commonly used antibiotics:

(1) The prevalent strains of N. meningitidis are, for all practical purposes, resistant to the inhibitory action of sulfonamides. Therefore, chemoprophylaxis with sulfonamides is no longer an effective method for preventing outbreaks of the disease (21). Although in vitro testing has shown the organisms to be sensitive, most of the commonly used antibiotics have been unsuccessful in curing the carrier state, apparently because antibiotic concentrations in the oropharaynx (or saliva) following "prophylaxis" doses are below the minimal inhibitory concentration needed (22). Two relatively new antibiotics, rifampin and minocycline, have shown some promise in eradicating the meningococcal carrier state, but their use should be limited because of rapid development of resistant mutants (rifampin) and toxicity (minocycline) (21).

(2) Over the past few decades the penicillin concentration required to inhibit in vitro growth of N. gonorrheae has steadily increased. In the United States prior to 1955 only 0.6% of tested strains required more than 0.05 units penicillin/ml for inhibition. By 1965 this figure had increased to 42.0% and in 1969 to 65.0% (23). Gonorrhea can still be treated with penicillin, but the dose required has increased to 4.8 million units (intramuscularly), given concomitantly with probenecid, 1 gram by mouth. In addition to their relative insensitivity to penicillin, many gonococcal strains are less sensitive to tetracycline and erythromycin in vitro and in vivo.

Although extrachromosomal plasmid DNA has been demonstrated in N. gonorrheae, there is as yet no firm evidence that resistance can be transferred by means of R factors. Recent studies, however, indicate that transfer of antibiotic resistance in gonococci can be accomplished by extracellular DNA liberated into the broth culture (24). Whether the DNA is plasmid or chromosomal in origin is unknown.

Immunology of Neisserial Infections

Antigens

The immunologically relevant antigens of Neisseriae are found on the bacterial surface and have been increasingly well characterized, particularly those of the meningococcus. The cell wall of N. meningitidis contains proteins and lipopolysaccharides (endotoxin) and is surrounded by an amorphous capsule of polysaccharide. These three components can be extracted together from the organism in the form of a "native complex," which is also released into culture fluid during bacterial growth (25). A similar complex has also been identified in human blood during systemic infection (26).

The capsular polysaccharides of N. meningitidis provide the basis for grouping these organisms. The serogroups and the chemical structure of their capsular polysaccharides, where known, are listed in Table III. These polysaccharides are immunogenic when presented on the intact bacterium in both man and rabbits, and highly specific antibodies raised to them in rabbits are utilized in a simple bacterial agglutination test to determine the serogroup of a given isolate.

The capsules are considered to have antiphagocytic properties and to provide a virulence factor for the organism. In the absence of a satisfactory animal model perhaps the best evidence for virulence related to the presence of a capsule is the fact that almost all blood and CSF isolates can be identified by serogrouping techniques. Of 1745 such cultures only four were nongroupable (34). In carrier surveys a much greater proportion of meningococci were nongroupable (9).

When isolated and purified, the capsular polysaccharides of group A and group C are immunogenic for man, but not rabbits, and have been shown to provide a highly protective vaccine against strains of these serogroups (35, 36). Protection is provided through complement-mediated bacteriolysis initiated by specific antibodies (bactericidal antibodies) which persist for at least five years following a single parenteral injection of vaccine (37). Serogroup B polysaccharide has not proved immunogenic in man (38).

Crude extracts of strains of N. meningitidis also contain many other broadly cross-reacting antigens, some of which may be important in the development of natural immunity by eliciting bactericidal antibodies. Using selectivity absorbed rabbit antisera and a bactericidal assay, meningococci have been classified into subtypes based upon subcapsular "factors" or antigens, which are independent of their capsular serogroup and can be demonstrated using antisera raised to both group B and group C organisms (39, 40). Some of the responsible antigens have been identified as protein in nature and all are thought to reside within the "native complex" of cell wall origin. Immuniza-

TABLE III

Chemical Characterization of Capsular Polysaccharides of N. meningitidis

Serogroup	Chemistry	Reference
A	N-acetyl, O-acetyl, D-mannosamine phosphate	(27)
B	N-acetyl neuraminic acid	(28)
C	N-acetyl, O-acetyl neuraminic acid	(28)
X	2-acetamido-2-deoxy-D-glucose 4-phosphate	(29, 30)
Y	Glucose	(31)
Bo*	Glucose 50%; neuraminic acid 50%	(32)
Z	Not characterized	
29E	3-deoxy-D-*manno*-octulosonic acid (53%) 2-acetamido-2-deoxy-galactosamine (47%)	(33)
135	Not characterized	

* Y and Bo may designate the same serogroup. There is strong immunologic cross reaction between them and they are usually used interchangeably. The presence of neuraminic acid in their capsules is a matter of dispute.

tion of rabbits with purified cell wall protein antigens from either serogroup B or C meningococci induces formation of bactericidal antibodies which lyse appropriate organisms of both homologous and heterologous serogroups (25). Thus, meningococcal cell wall proteins may be regarded as important antigens in eliciting immune responses, at least in some mammalian hosts, which may provide rather broad immunity against the species.

Lipopolysaccharides (LPS or endotoxin) purified from meningococci are highly toxic, being as potent a lethal toxin in mice as the LPS from E. coli or S. typhimurium, and their ability to elicit the local or dermal Shwartzman reaction in rabbits is 5- to 10-fold greater than that of enteric LPS (41). These observations are consistent with clinical experience which has shown that meningococcal septicemia is often associated with purpura, which is seldom observed in coliform septicemia. Although a role for LPS antibodies in immunity to meningococcal infection has yet to be shown, recent studies (42) have demonstrated an LPS antibody response in humans during the course of systemic disease. These hemagglutinating antibodies directed against LPS (as well as those directed against protein) antigens showed no correlation with serogroup or serotype of the infecting strains.

Similar methodology for separation and purification of the outer membrane or "native complex" of N. gonorrhoeae has also been described (43), and further clarification of its immunochemistry is in progress. Unlike the meningococcus, a well defined capsular polysaccharide has not been identified on the gonococcus. Yet in the past (44), and again recently (45), a few reports have described the extraction of polysaccharide antigens from gonococci, and a capsular structure has been observed in fresh gonococcal isolates by light and electron microscopy in our laboratory (13).

Anti-gonococcal bactericidal antibodies analogous to those directed against the meningococcus have been identified in both human and rabbit sera, but their role, if any, in resistance to gonococcal dissemination or local inflammation is unknown. In immune rabbit antisera they have been shown to be directed against both LPS and protein antigens and to be cross-reactive with similar antigens of the meningococcus (4). Indeed, these two very similar organisms may share not only surface antigens but susceptibility to the same systemic host defenses.

The discovery that gonococci of colonial types 1 and 2 have pili on their surface (46) has led to a series of investigations of this morphologically distinct structure. It has been suggested that they play a significant role in the attachment of gonococci to epithelial cells (47) and that they may provide the organism with some resistance to phagocytosis. The pili are antigenic and antibodies raised to them inhibit both agglutination of erythrocytes and attachment to squamous buccal cells by piliated gonococci (48). Thus, it is thought that pili may play an important role in the early events of infection by facilitating attachment of gonococci to susceptible mucosal epithelial cells and that inhibition of this event by antibody might well prevent a true infection.

One further observation about Neisserial antigens will be discussed in very brief terms. A number of bacterial species as diverse as Bacillus pumilis and E. coli have been found to contain antigens which cross react with antibody raised against meningococci (49, 50). These bacterial strains contain antigens similar, and in some cases identical, to polysaccharides of meningococcal serogroups A, B and C, as well as to subcapsular antigens which have not been further defined. The suggestion has been made that exposure to these cross reactive antigens may result in the development of a natural immunity to meningococcal infection. It is of some interest, then, that the N-acetyl neuraminic acid polymer purified from the Kl capsular polysaccharide of a strain of E. coli failed to elicit an antibody response in human volunteers (51). This Kl polysaccharide was shown to be identical to the group B meningococcal capsular polysaccharide, which also failed to induce an antibody response in humans.

Immunodiagnosis

1. *Presence of antigens in body fluids and tissues*. Detection of an intact organism in excretions, secretions or in tissues of the inflamed target organ is extremely useful in establishing that organism as the causative agent of the disease. When Gram's stain yields Gram-negative diplococci in smears of cerebrospinal fluid or of a petechial skin lesion, a diagnosis of meningococcal disease is highly likely, N. gonorrheae being a distinct second possibility. Similarly, the findings of Gram-negative diplococci within polymorphonuclear leukocytes of the urethral discharge of a male or in joint fluid of a female makes the diagnosis of gonococcal disease highly likely. Gram's stain of exudate from the female genital tract is less significant in terms of etiologic diagnosis since other morphologically similar Neisseriaceae may be present. Immunofluorescent staining procedures utilizing antibodies raised in rabbits to whole gonococci can improve diagnostic accuracy in the latter situation. The "Quellung" reaction, in which specific antisera cause visible capsular swelling of encapsulated organisms, such as the meningococcus, has not been utilized very much in recent years as compared to times past when specific antisera were used in treatment of pneumonia and septicemia.

The detection of certain microbial antigens is equally useful in demonstrating the etiology of infections. The development of counter-immunoelectrophoresis (CIE) has made it possible to detect minute amounts of microbial antigens in infected body fluids very rapidly. Hoffman and Edwards have shown that CIE can detect group C meningococcal polysaccharide in concentrations of 0.012 micrograms/ml and demonstrated this antigen in 29% of acute sera tested and 70% of CSF specimens from patients with group C meningococcal disease (52). The presence and concentration of antigen in serum or CSF correlated with the severity of the clinical illness. Other investigators have shown that CIE is highly effective and specific in diagnosing meningococcal, pneumococcal and Hemophilus influenzae type b meningitis (53); and Greenwood et al (54) even found CIE to be better than culture and Gram's stain in diagnosing group A meningococcal meningitis. The test can provide positive results within an hour and, therefore, can be extremely helpful in selecting appropriate therapy. Diagnosis by CIE depends upon the presence of highly specific soluble bacterial antigens in the body fluid tested and its sensitivity is highly dependent upon the potency of the antiserum use. Potent, specific, group B meningococcal antisera are particularly scarce. Though positive results occur frequently in meningococcal infections they have not as yet been reported in gonococcal infections.

Being Gram-negative, the Neisseriae possess a lipopolysaccharide endotoxin (vide supra) whose presence in bodily fluids can be detected by the Limulus lysate gelation assay. This assay is highly sensitive and specific for endotoxin and patients with meningococcal meningitis have been shown to have measurable quantities of LPS in their CSF. Hemophilis influenzae also possesses endotoxin, however, which has been detected by the Limulus assay in the CSF of patients with meningitis due to this Gram-negative organism (55). The Limulus assay, therefore, shows great promise in distinguishing Gram-negative meningitis from Gram-positive, but not in distinguishing meningococcal from that caused by other Gram-negative bacteria.

2. *Antibody assays.* A large number of assays have been developed to measure serum antibodies to N. meningitidis (Table IV). The use and interpretation of the various antibody tests in studies of the meningococcus is dependent upon a number of factors. For example, carrier and systemic infections usually induce antibody responses to many different antigens and the test selected will depend upon the problem under investigation. If the problem is merely to document that the illness was associated with a Neisserial infection, the CF test is the simplest and most broadly reactive, but will not distinguish between members of the genus. If the causative organism is known to be a meningococcus of a certain serogroup, hemagglutination (HA)

TABLE IV

Assays for Meningococcal Antibodies in Humans

Assays	Antigen specificity	Immunoglobulin detected	Reference
Passive Hemagglutination	Polysaccharide	IgM (predominant)	(56)
	Protein		(42)
	Lipopolysaccharide		(42)
Latex agglutination	Polysaccharide	IgM (probable)	(57)
Complement-fixation	Broad crossing among meningococcal serogroups	IgM, IgG (probable)	(56, 58)
Precipitin (double diffusion in gel)	Polysaccharide	All classes	(56)
Immunofluorescence	Whole organism	IgM, IgG, IgA	(7)
Bactericidal	Whole organism with serogroup and also subtype specificities	IgM, IgG, IgA*	(7, 10)
Opsonization	Polysaccharide	IgG, IgM	(59)
Antigen binding (radioactive)	Polysaccharide	All classes	(60)
	Other antigens	IgG, IgM, IgA	(61)

* IgA binds to organism but does not lyse it, causing diminished effectiveness of IgM and IgG.

and latex fixation (LF) would be the simplest assays to perform and would preferentially detect early antibodies of the IgM class which are associated with recent infection. Since meningococci of serogroups B and C presently account for about 95% of all meningococcal infections in the United States with group Y accounting for most of the remainder, one need routinely test for only these three serogroups plus group A, which, though currently absent from the US, has been the predominant cause of large epidemics in the past. Indeed, group A meningococcus is currently causing a widespread epidemic in Brazil (along with group C) and is present in native Indian populations in Canada. Its reappearance in the US is probably only a matter of time. The human immune response to group B polysaccharide is muted, as compared to group A or C responses and, therefore, a false negative HA test for group B infection may occur. An HA test performed on a single serum may also be misleading due to preexisting natural antibody to the antigen.

A primary radioactive antigen-binding assay (RABA) utilizing a modification of the Farr technique is a much more sensitive and direct way of measuring antipolysaccharide antibodies of all three major classes. In studies of polysaccharide immunization of adults, HA is simple and sensitive; however, the low titer responses of infants and young children require use of RABA for detection and quantitation (62).

Bactericidal (BC) and opsonization assays are the preferred tests to relate antibody results to immune status; of the two assays, BC appears to be the more sensitive and reproducible and its relevance to immune status has been more clearly defined. However, the complexities and variables in the BC test (bacterial culture growth medium and duration, inoculum size, source of complement) preclude its use in any but a research laboratory. The antigenic characteristics of the BC test strain are critical in interpretations of results since most human sera contain a variety of antibodies directed against serogroup and serotype antigenic determinants. Added to this array of reactions is the possibility that IgA in certain sera may inhibit the bactericidal activity of IgG and IgM antibodies (10).

Gonococcal serology is in a more primitive stage of development as compared to meningococcal serology. The prime reason for this is the lack of definitive knowledge of the relevant antigens of N. gonorrhoeae. A large variety of serologic assays has been reported including hemagglutination and other agglutination techniques, immunofluorescence and complement fixation, with similar results reported for each: namely, positive results in 80–90% of infected women and 25–66% of culture positive males. Uninfected control subjects have a 5–10% incidence of antibody reactivity (53). Even local secretory IgA antibodies are present in a large proportion of infected subjects (64). Buchanan et al (65) have reported on serologic studies using a purified preparation of gonococcal pili as a radioactive antigen. Mean titers of infected males and females were significantly elevated when compared to mean values of controls. However, only 50% of acutely infected symptomatic males had titers above the range of controls, while 89% of asymptomatic infected females had titers higher than the highest control subjects. Thus, this assay does not appear to be significantly better as a diagnostic tool than earlier, simpler tests. None of the serologic assays for gonorrhea described to date appears to have any relationship to a state of immunity (66).

Conclusion

In summary, the Neisseriae are a rather homogeneous group of Gram-negative organisms that share an ecologic niche with humans, residing on human mucous membranes in partial symbiosis, but capable of rapid proliferation leading to exudative inflammation. For reasons that are only beginning to be understood, two of their number, the meningococcus and the gonococcus, may infrequently escape from the host restraints that normally delimit their range, invade the blood stream and produce disease which may be fulminantly fatal or chronically disabling. With reasonable attention to their growth requirements they are neither hard to recover on culture, nor difficult to identify. Their immunochemistry is under intensive study and its understanding has already led to the partial control of N. meningitidis by a vaccine consisting of purified capsular polysaccharide material. Despite this, serodiagnosis has not yet proved clinically feasible or timely, and the diagnosis of disease produced by them rests upon a proper clinical and epidemiological setting, confirmed by culture results. Their treatment by a variety of common antibiotics, including penicillin, remains satisfactory despite emergence of selective and incomplete resistance.

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Abnormal Forms of Bacteria

VICTOR LORIAN, M.D.

The present paper reviews aberrant forms of bacteria that occur with exposure to subinhibitory concentrations of antibiotic. The abnormal forms may resemble and be mistaken for other species. Their presence in wounds or as isolates from blood and body fluids may indicate previous, unsuspected therapy with antibiotics.

Introduction

Approximately one-third of cultures processed in a hospital laboratory are from patients who are receiving antibiotic therapy at the time the culture is taken (9).

Case 1 (Blood): A 42-year-old woman, admitted for high fever, weakness, and intense headaches, had a temperature of 102.5°F. and leucocyte count of 18,000. She was started immediately on ampicillin intravenously, 1 Gm every 4 hr; blood was drawn for culture a few hours later. The culture became positive at 48 hr and Enterobacter cloacae sensitive to ampicillin was isolated.

Gram-negative filaments 20 to 100 microns long were found in the culture. The Wright stain gave a much better tinctorial contrast than did the Gram stain (26; Fig. 1).

Case 2 (Urine): An 84-year-old man, hospitalized because of complete heart block, developed multiple infections 3 days after admission. Six different species of bacteria were cultured repeatedly from the sites of infection: a decubitus ulcer, the respiratory system, the urinary tract, and the blood (26). The urine sediment showed many filaments and the culture showed E. coli (Fig. 2).

Antibiotic treatment for 10 days preceding the urine culture consisted of chloramphenicol, 3 Gm daily for 7 days, followed by penicillin, 3,000,000 U daily and ampicillin, 6 Gm daily. At the time the urine culture was obtained, only ampicillin was being given.

The effects of subinhibitory concentrations of penicillin on the morphology of bacteria in vitro were observed shortly after the discovery of penicillin. Gram-negative rods, particularly, grew into long filaments (5, 11), while staphylococci showed great swelling and enlargement in diameter (11, 32). Other studies with different drugs and species revealed a continuum of

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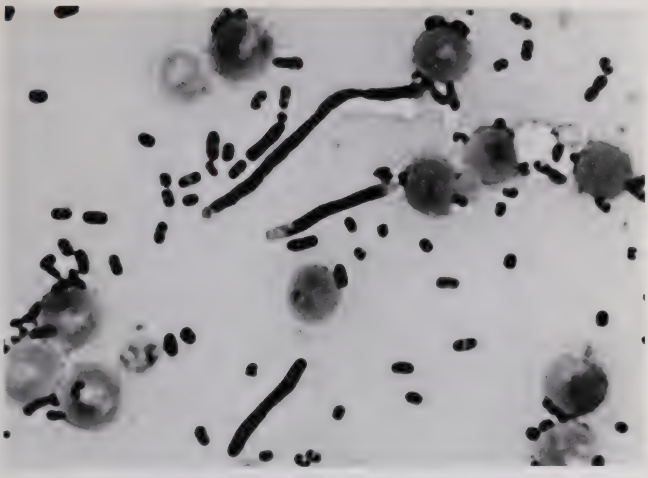

FIG. 1. Filamentous form (32 mμ long) of Enterobacter cloacae from a blood culture. Wright stain (1,250×).

aberrant forms that ranged from filaments to bulbous and spindle-shaped forms to enlarged and rounded "spheroplasts" (2, 10, 15, 20). The number and kind of abnormal forms present in a culture varied with the species, with the antibiotic used, and with the concentration of antibiotic in the medium (15, 20).

Spheroplasts, which undergo lysis, represent the terminal end of the continuum of aberrant forms that can be induced by subinhibitory concentrations of antibiotics. Spheroplasts also can be induced in hypertonic media, as can abnormal forms of bacteria with deficient cell walls. These so-called "L-forms" may resemble Mycoplasma and may revert to the parental form under appropriate conditions. Spheroplasts and "L-forms" are synonymous when referring to Gram-negative bacteria (27).

Forms of bacteria morphologically similar to L-forms have been isolated from the blood, body fluids and exudates of patients who were, and who were not, receiving treatment with antibiotics (27, 35).

Clinical studies have suggested that L-forms could be behind the persistence in the organism of bacteria after treatment with antibiotics that attack the cell wall. It is thought that the transformation of the bacillus into a drug-resistant L-form may occur spontaneously (34), after exposure to antibodies (6), or in response to antibiotic therapy (27, 35). The aberrant form, which may or may not be clinically significant, may revert to the pathogenic parent form and cause a clinical episode or a relapse in disease (27, 35).

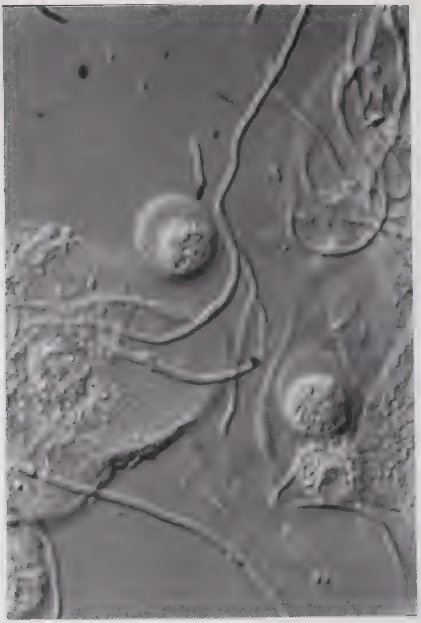

FIG. 2. Filament of E. coli in urine sediment. Smith phase contrast (1,250×).

Filaments are the initial response of Gram-negative or Gram-variable bacteria to concentrations of antibiotic that do not inhibit growth or division of the nuclear apparatus (10, 18), but that block division of the cytoplasm, apparently by inhibiting the enzymes that initiate septation (3). Filamentation has been induced at dilutions 8 or 10 times, sometimes even 30 times lower than the minimum inhibitory concentration (MIC) (11).

Because subinhibitory concentrations of antibiotics can act synergistically or antagonistically with other drugs, it is of practical value to define antibacterial activity at low concentrations. The minimum antibiotic concentration (MAC) is the lowest concentration of an antibacterial agent that would affect the rate of growth or the structure of bacteria or both (22, 23, 25).

The MAC varies with different strains and different drugs. Concentrations higher than the MAC may induce forms other than filaments (15), while the number of forms that occur within a given strain at a particular antibiotic concentration may vary widely (20).

Morphology

The following description, unless specified otherwise, is based on data presented by Lorian and Atkinson (24).

Penicillin and ampicillin at subinhibitory concentrations block division by inhibiting growth of a septum at the cross wall sites. There, the mucopeptide layer of the cell wall has undergone hydrolysis in order to accomodate the mucopeptides destined for septum formation (3). A concentration of ⅙ the MIC of ampicillin produced filaments 30–90 mμ long in Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, and Salmonella typhimurium (Figs. 3 and 4). At ¼ the MIC of ampicillin the same strains produced oval globules. Penicillin caused filamentation in Proteus vulgaris, Vibrio cholerae, Salmonella typhimurium, and the formation of threads with bulbs in Pseudomonas pyocyanea (10). Penicillin induced filaments in E. coli at ⅒ the MIC (20). Sulfathiozol induced filamentation in Bacterium anitratum, Lactobacillus vaginalis, and Pasteurella tuberculosis (2). Nalidixic acid, at ¼ of the MIC produced filaments 20 to 50 mμ long in E. coli, P. mirabilis, and K.

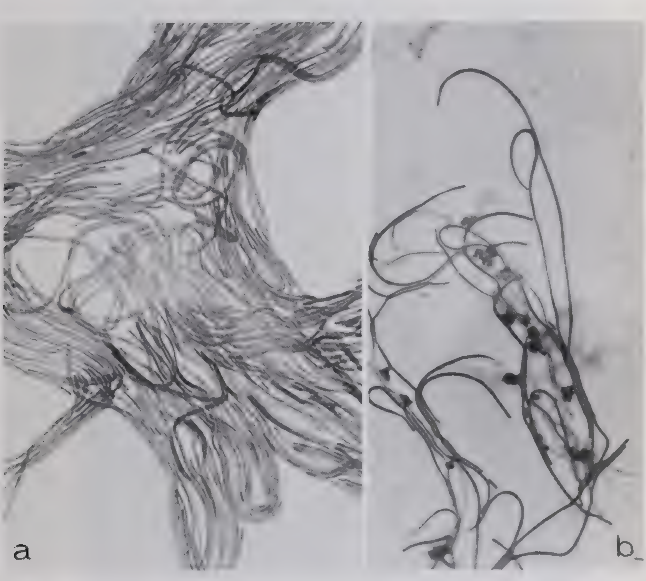

FIG. 3. a) Filaments of E. coli exposed to ampicillin and b) filaments with blebs of E. coli exposed to nitrofurantoin. Gram stain (1,250×).

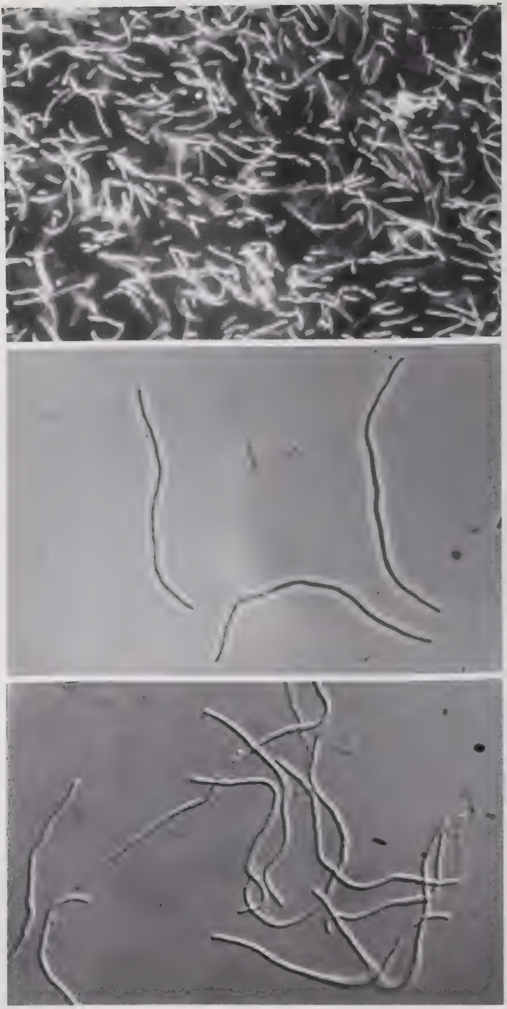

FIG. 4. Filaments of E. coli after exposure to ampicillin. *Upper:* Dark field (300×). *Middle:* Phase contrast (1,250×). *Lower:* Smith phase contrast (1,250×).

Pneumoniae (13). Nitrofurantoin at 1/8 and at 1/4 the MIC produced in E. coli filaments with blebs which resembled micelia with spores (25).

Tetracycline, chloramphenicol, and gentamycin, at 1/10 to 1/2 the MIC, caused elongation and bipolar staining of E. coli and S. typhimurium. Kanamycin at 1/10 the MIC produced similar results in E. coli (20; Fig. 5). Streptomycin after 7 hours produced in E. coli an elongated rod with a centrally translucent, dark subterminal granule which resembled a clostridium with fully developed spore (30).

Electron microscopy shows the effects on ribosome distribution of agents

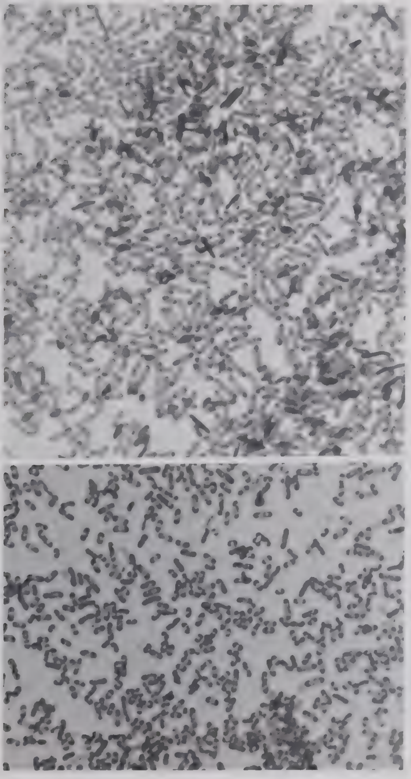

FIG. 5. Salmonella typhimurium, elongated forms after exposure to tetracycline (upper) and bipolar staining after exposure to gentamycin (lower). Gram stain. (1,250×).

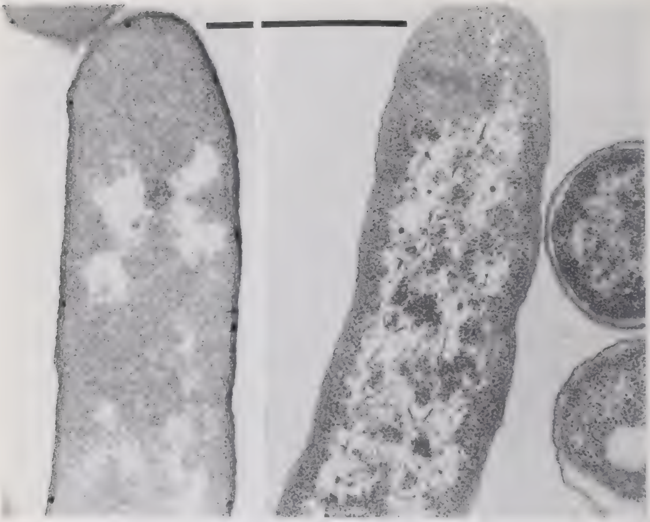

FIG. 6. Salmonella typhimurium: control (left) and exposed to gentamicin ($^1/_2$ the MIC; right). Note diffuse decrease in the number of ribosomes per square μm, except at the end of the cell (50,000×). Bar = 1 μm (Fig. 13, p. 684, *in* Lorian, V. and Atkinson, B., 1975. Abnormal Forms of Bacteria Produced by Antibiotics, Am J Clin Path 64(15):678. Reprinted with the permission of the publisher).

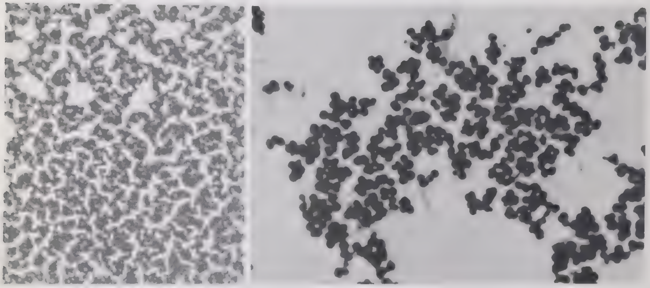

FIG. 7. Left. Staphylococcus aureus grown on drug-free agar (control). Gram stain (1,250×). Right. Staphylococcus aureus grown on agar containing penicillin ($^1/_3$ the MIC): large cells were produced. Gram stain (1,250×).

that act within the cell. Gentamicin caused bipolar staining in S. typhimurium. Electron microscopy of S. typhimurium exposed to ½ the MIC of gentamicin showed a diffuse thinning in the distribution of ribosomes except at the two ends of the cell (Fig. 6). Tetracycline produced E. coli of a granular appearance. Electron microscopy of E. coli that had been exposed to ½ the MIC of tetracycline produced islands without ribosomes which were surrounded by regions of high ribosome density. Electron microscopy of S. typhimurium exposed to ½ the MIC of chloramphenicol showed a reduction in the number of ribosomes at the center of the cell, as has been observed at higher concentrations (12). Chloramphenicol, possibly by inactivating (rather than by blocking synthesis of) an autolysin in the cell wall (3), promotes the formation of bulges with elongation (20).

In Gram-positive cocci, the mucopeptide component of the cell wall is greater than it is in Gram-negative bacteria (31). Subinhibitory concentrations of antibiotics differently affect the cell walls and lead to spherical enlargement rather than to the formation of filaments (11, 32).

Staphylococcus aureus exposed to ⅒ the MIC of penicillin showed clusters of ballooning cells with variable shapes, some of which showed blebs (20).

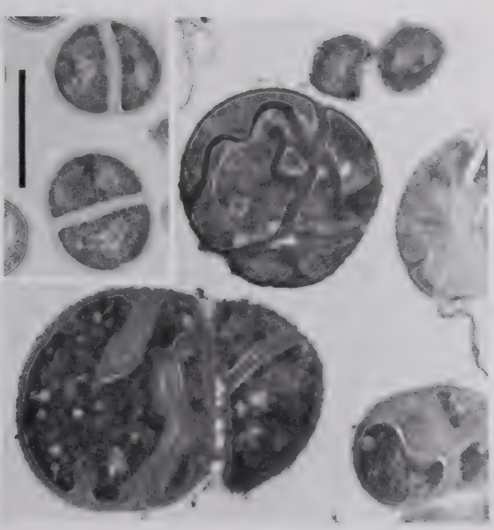

FIG. 8. Staphylococcus aureus grown on agar containing ⅓ the MIC of penicillin. Insert (upper left) shows control cells. Electron micrograph (30,000×). Bar = 1 μm (Fig. 2, p. 1050 *in* Lorian, V., 1975. Some Effects of Subinhibitory Concentrations of Antibiotics on Bacteria, Bull NY Acad Med 51(9):1046. Reprinted with the permission of the publisher).

Exposed to 1/3 the MIC of penicillin, the cells at least doubled in size and retained more crystal violet, which indicated an increase in the mucopeptide content of the cell wall (31; Fig. 7). Electron microscopy of Staphylococcus aureus exposed to penicillin showed, in addition to oval and other irregular shapes, thick and numerous cross walls (Fig. 8). Increased material in the cross walls could account for the increase in cell size that was observed (7). At the MIC, balloons with blebs or raspberry shapes occur, followed at a higher concentration by the formation of spheroplasts and lysis (20).

Streptococcus lactis exposed to benzylpenicillin formed Gram-variable rods (7). Streptococcus pneumoniae exposed to 1/2 the MIC of methicillin formed Gram-variable rods and globules or "tennis racket" shapes (Fig. 9). Streptococci group D which had been exposed to 1/2 the MIC of penicillin showed elongated forms and abnormal cross walls (Fig. 10). In Streptococcus pyogenes penicillin produced swelling, "raspberry" shapes, and increased length of chains (11).

Neisseria gonorrhoeae, exposed to 1/4 the MIC of penicillin, formed connected tetrads or conglomerates of 8 or 16 organisms (Fig. 11). Electron microscopy revealed septa that were abnormally wide (Fig. 12).

The increased cell size seen in staphylococci after treatment with a subinhibitory concentration of penicillin was previously ascribed to the inhibition of cytoplasmic division (32). The abnormally thick septa revealed

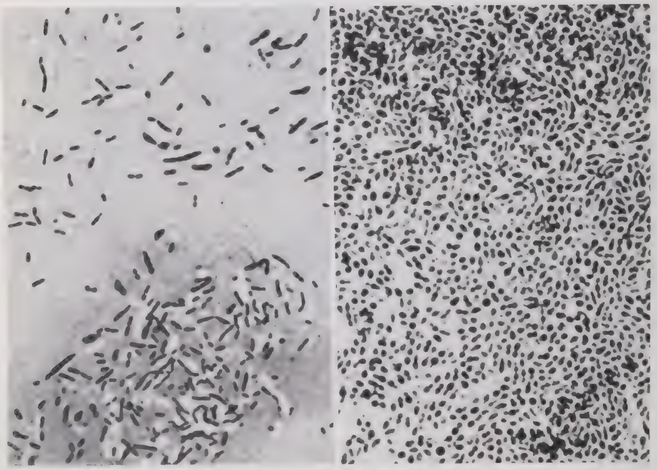

Fig. 9. Left. Pneumococci exposed to methicillin (1/4 the MIC) for 7 hours produced rod-like forms. Some organisms of abnormal shape can also be seen. Right. Pneumococci exposed for 15 hours to methicillin. Most organisms turned into globules. Gram stain (1,250×).

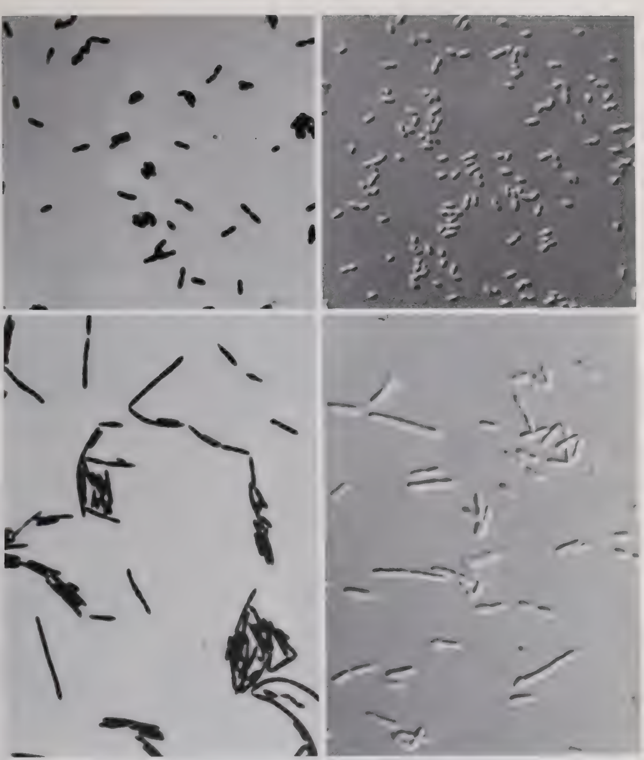

FIG. 10. Streptococci group D: control (upper) and filamentous forms (lower) after exposure to penicillin. *Left:* Gram stain. (1,250×). *Right.* Smith phase contrast. (1,250×).

by electron microscopy of swollen forms of staphylococci, the rods of pneumococci, and the tetrads formed by gonococci may hold together cells that are otherwise divided. In staphylococci, penicillin appears to inhibit lysis of the cross wall, but not to inhibit cell division. The inhibition of lysis of the cross wall by penicillin is reversible: cells that are normal in appearance and in growth rate separate from clusters of unseparated staphylococci when they are incubated on drug-free media (23).

Mycobacterium tuberculosis may lose its acid-fastness when exposed to INH (21). It may therefore be present in the sputum of a patient who has been treated with INH when a Ziehl-Neelsen stain suddenly gives negative results.

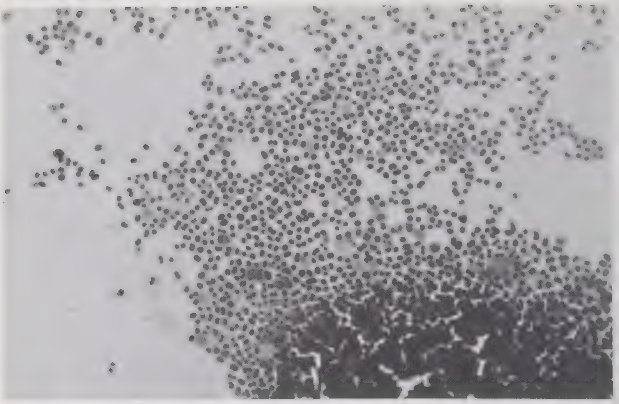

FIG. 11. Gonococci exposed to penicillin—very large cells and globules. Gram stain. (1,250×).

Discussion

Abnormal forms of bacteria have been isolated in vivo and produced in vitro. Filaments and other forms that precede spheroplast formation have been isolated before and after treatment with antibiotics. Spheroplasts in turn may precede the formation of wall-less forms or themselves be an endpoint of morphologic change in response to antibodies or antibiotics. Spheroplasts are also an endpoint in the response of bacteria to subinhibitory concentrations of β-lactam antibiotics.

While the adaptive value of spheroplasts and other L-forms has been recognized (17), little can be said about the possible adaptive value of the aberrant forms that are induced in vitro by subinhibitory amounts of antibiotic. Like L-forms, these forms have been isolated, and unlike L-forms, occasionally seen, in vivo.

Filaments and other abnormal walled forms have been isolated from superficial wounds, from deep suppuration, and from the oral mucosa of normal adults (2). Filaments of bacteroides and giant staphylococci have been found in wounds, and rod-like pneumococci have been seen in sputum (24). A bipolar stained form of K. pneumoniae was found in pus from cellulitis of a patient treated with gentamicin (29). Filaments have been present in the urine of patients who were and of patients who were not receiving antibiotics (1, 16). Filaments of enterobacteria have been observed in the blood of patients in whom antibacterial therapy had been recently initiated (26).

Spheroplasts of staphylococci, enterococci, and Gram-negative rods have been isolated from heart muscle biopsy of patients with chronic rheumatic heart disease (14), and protoplasts (L-forms) of streptococci have been isolated from kidney biopsy specimens (19).

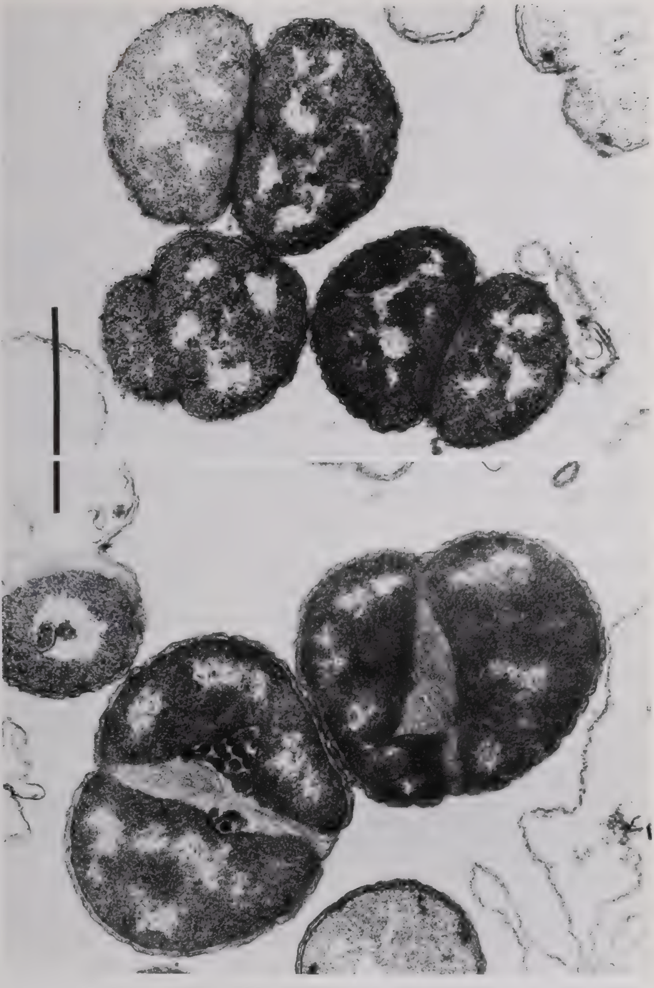

FIG. 12. Gonococci, control (upper) and the same organism exposed to ¼ the MIC of penicillin. Note that the cells are larger and the cross wall is much wider and of more irregular shape than in the control (50,000×). Bar = 1 μm. (Fig. 16 *in* Lorian, V. and Atkinson, B., 1975.

Possibly the clustering of cocci and the formation of filaments, which decreases the surface area for each cell, allows persistence under a wider range of environmental conditions, and the establishment of a colony of bacteria when environmental conditions improve. It is also possible that the filaments of Gram-negative bacteria and the clusters of cocci are differently affected by the various defense mechanisms because they are damaged bacteria.

Changes in surface configuration, such as the formation of blebs which may roughen the surface, may render bacteria more phagocytable (33). Also, the reduced surface area that occurs in filaments and in clustered cocci, could limit the extent of motility (10).

Factors other than exposure to antibiotics can cause considerable variation in the morphology of bacteria. In Gram-negative bacilli grown in a rich culture medium, the rate of growth will accelerate, length will increase (28), and the number of ribosomes will increase (8).

Summary

Filaments and other forms of bacteria which are induced in vitro by subinhibitory concentrations of antibiotic may resemble other species. They may precede the development of wall-defective forms or of spheroplasts which undergo lysis. Their presence in vivo as isolated from blood and body fluids or exudates may indicate the presence of subinhibitory concentrations of antibiotic resulting from previous or unsuspected therapy.

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Laboratory Diagnosis of Anaerobic Infections

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Pathogenesis of Anaerobic Infections

Virtually all anaerobic infections arise endogenously. Anaerobic bacteria are found as part of the normal flora of the skin and are prevalent as indigenous flora on all mucous membrane surfaces. In many of these locations, they outnumber aerobic or facultative bacteria. For example, in the mouth and upper respiratory tract, anaerobes outnumber facultative forms ten to one. In the gastrointestinal tract, anaerobes outnumber facultative forms one thousand to one. Thus, Escherichia coli, a well known member of the normal colonic flora, actually constitutes only 0.1% of this flora.

Under certain circumstances (trauma, tumors arising at the mucosal surface, etc.) anaerobes from the indigenous flora have an opportunity to penetrate tissues and thus to set up infection. Occasionally, as in aspiration pneumonia, anaerobic bacteria from a site of normal carriage may be carried into another area normally free of bacteria and set up an infection (1). Poor blood supply and tissue necrosis lower the oxidation-reduction potential and favor the growth of anaerobic bacteria. Prior infection with aerobic or facultative bacteria may also make conditions more favorable for growth of anaerobic bacteria. As with infection due to aerobic or facultative bacteria, various conditions in which host defense mechanisms are impaired and prior antimicrobial therapy to which the infecting organism is resistant may pave the way for anaerobic infection. Involvement of anaerobic bacteria in infectious processes may also depend on resistance of certain of these organisms to normal body defense mechanisms. Thus, there is evidence that certain subspecies of Bacteroides fragilis are more commonly involved in infection than others because of their resistance to normal bactericidal activity of serum (2). Anaerobic bacteria may also produce toxins and certain other substances such as hyaluridonase, proteinase, etc., which account for virulence.

Types of Infections Involving Anaerobes

Anaerobic bacteria may be involved in any type of infection which can be caused by bacteria. All organs and tissues of the body are susceptible to infection with these organisms. Table I lists a number of infections in which anaerobes are found with some frequency.

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Since the major sources of anaerobes in the body are the various mucosal surfaces, most infections involving these bacteria arise at or near mucosal surfaces. Differences in distribution of specific anaerobes throughout the body's normal flora account for involvement of different species in particular infections. Thus, intra-abdominal infections, most of which arise from the bowel, involve Bacteroides fragilis very commonly; this organism is the dominant member of the normal human colonic flora. Infections with anaerobic streptococci involve structures of the mouth and upper respiratory tract and the female genital tract much more commonly, reflecting the fact that these organisms are more prevalent in those areas normally.

Clues to Anaerobic Infection

Tables II and III list certain clinical and bacteriologic clues to anaerobic infection. The foul odor of discharges is by far the most characteristic and definitive clue to anaerobic infection. This odor, falsely attributed to E. coli, is not always found with anaerobic infections (certain organisms do not produce foul-smelling products and, at times, there is no communication between the site of infection and the outside of the body) but is a definite indication that anaerobes are involved in an infectious process when it is present. Certain of the clinical clues, of course, are nonspecific, but combinations of two or more (tissue necrosis and gas in tissues, for example) would be very suggestive of the possibility of anaerobic infection. As will be noted later, anaerobic bacteria are

TABLE 1

Some Infections in Which Anaerobes Are Important Pathogens

Bacteremia
Brain abscess
Otogenic meningitis, extradural or subdural empyema
Chronic otitis media
Dental infections
Pneumonia secondary to obstructive process
Aspiration pneumonia
Lung abscess
Bronchiectasis
Thoracic empyema
Liver abscess
Pylephlebitis
Peritonitis
Diverticulitis
Subphrenic abscess and other intraabdominal abscesses
Wound infections following bowel surgery or trauma
Puerperal sepsis
Postabortal sepsis
Endometritis
Tubo-ovarian abscess and other gynecologic infections
Perirectal abscess
Gas-forming cellulitis

TABLE 2

Clinical Clues to Anaerobic Infection

Foul-smelling discharge
Location of infection in proximity to a mucosal surface
Necrotic tissue, gangrene; pseudomembrane formation
Gas in tissues or discharges
Endocarditis with negative routine blood cultures
Infection associated with malignancy or other process resulting in tissue destruction
Infection related to the use of aminoglycosides (oral, parenteral, or topical)
Septic thrombophlebitis
Bacteremic picture with jaundice
Infection following human or other bites
Black discoloration of blood-containing exudates; these exudates may fluoresce red under ultraviolet light (B. melaninogenicus infections)
Presence of sulfur granules in discharges (actinomycosis)
Clinical setting suggestive of anaerobic infection (e.g., septic abortion, infection following gastrointestinal surgery)

TABLE 3

Bacteriologic Clues to Anaerobic Infection

Unique morphology on Gram stain (Figs. 1, 3 through 5)
Failure of the organisms seen on Gram stain of original exudate to grow aerobically. (Failure to obtain growth in fluid thioglycollate medium is not adequate assurance that anaerobes are not present.)
Growth in anaerobic zone of fluid media or of agar deeps
Growth anaerobically on media containing 100 μg of kanamycin, neomycin, or paromomycin per milliliter (or a medium also containing 7.5 μg of vancomycin per milliliter in the case of Gram-negative anaerobic bacilli)
Gas, foul odor in specimen or culture
Characteristic colonies grown anaerobically on agar plates (Fig. 2)
Young colonies of B. melaninogenicus may fluoresce red under ultraviolet light

usually quite resistant to aminoglycoside antimicrobial agents; hence, the infection related to the use of such agents.

Incidence of Anaerobes in Infection

Recently good data have become available on the incidence of anaerobic bacteria in various types of infections. In the study of Martin (3), the incidence of anaerobes was found to be 49% in a series of cultures of more than 14,000 specimens which yielded growth.

Several clinical studies have provided accurate data on the incidence of involvement of anaerobes in a variety of clinically significant infections. Heineman and Braude (4) have shown that about 90% of brain abscesses involve anaerobic bacteria, almost two-thirds of them involving anaerobes alone. In lung abscess, over 90% of properly obtained specimens will yield anaerobic bacteria, two-thirds of them in the absence of aerobic or facultative forms (5). In the case of aspiration pneumonia, again over 90% of specimens collected so as to bypass normal flora will contain anaerobic organisms, half of

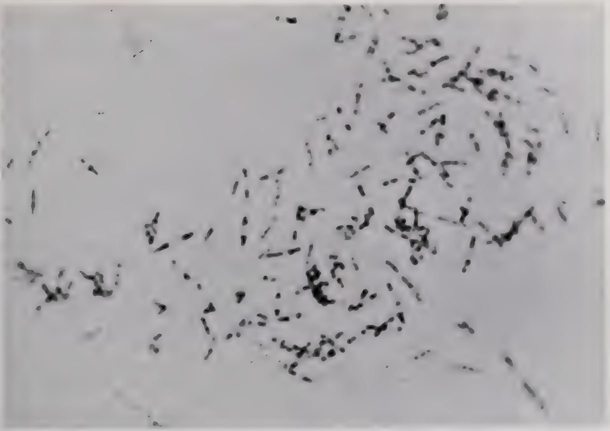

FIG. 1. Bacteroides fragilis, microscopic morphology. Note irregular staining which is often bi-polar ("safety-pin effect") and certain amount of pleomorphism. (From Center for Disease Control, Atlanta)

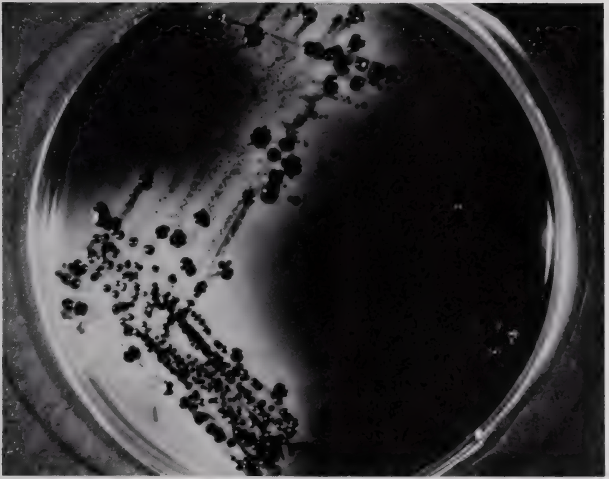

FIG. 2. Bacteroides melaninogenicus, colonial morphology. Note distinctive black pigmented colonies and clearing of blood from adjacent medium.

these to the exclusion of aerobic or facultative forms. In the case of aspiration pneumonia acquired in the community, rather than in the hospital, two-thirds of the anaerobes will be recovered in the absence of aerobic or facultative forms (1). In thoracic empyema not related to previous surgery, 75% of specimens yield anaerobes, approximately half of these in the absence of aerobic or facultative forms (6). Ninety-percent of cultures of material from intraabdominal infections yield anaerobic organisms, but here the incidence of anaerobes isolated alone is only 10% (7). In the case of liver abscess, approximately 50% of abscesses have shown anaerobes (8) but it is likely that with a prospective study and optimal bacteriologic procedures, the incidence would be significantly higher. In the study just cited (8), when anaerobes were isolated, they were found to the exclusion of aerobic or facultative organisms two-thirds of the time. In the case of female genital tract infections, the incidence of anaerobes among 200 patients with such infections compiled from three laboratories (9–12) was 72%, with anaerobes the exclusive isolates in 30% of cases.

Specimens to Culture for Anaerobes

Since anaerobes are involved commonly in a variety of infections and since all types of infections may involve anaerobes, all types of specimens should be cultured for these organisms. However, since anaerobes are so prevalent as normal flora, it is also important to keep in mind that no specimen which is contaminated with normal flora should be cultured for anaerobic organisms since the results will be meaningless and the laboratory will be burdened unnecessarily. Since anaerobes characteristically produce abscesses, it is important that all abscesses be cultured anaerobically. When clinical or bacteriologic clues suggesting anaerobic infection are present, it would be especially important to do anaerobic cultures.

The following types of specimens should *not* routinely be set up for anaerobic culture—throat culture, gingival swab, gastric contents, small bowel contents, feces, coughed sputum, voided urine and vaginal swabs. Any other specimen which may be "contaminated" with normal flora should also not be submitted for anaerobic culture. Exceptions will have to be made in certain instances. For example, in suspected "blind loop syndrome" quantitation of aerobic or facultative and anaerobic flora of the small bowel or afferent gastric loop contents may be important diagnostically and in guiding therapy.

Collection and Transport of Specimens

Proper specimen collection and transport are absolutely essential if reliable meaningful results are to be obtained on anaerobic culture. Ordinarily, these two important considerations will be the responsibility of the clinician. However, it may be necessary for the microbiologist to properly instruct the clinician in the importance of proper collection and transport and also regarding specific techniques which are appropriate.

In suspected anaerobic pulmonary infection, when empyema fluid is not available, percutanous transtracheal aspiration is ordinarily the procedure of

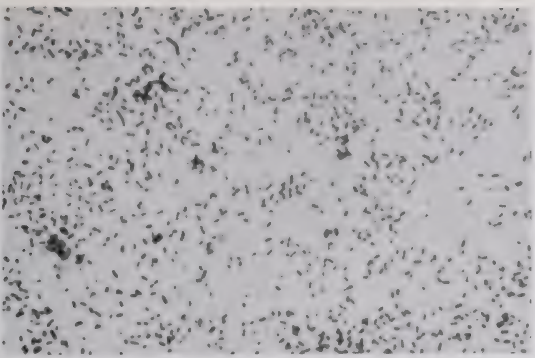

FIG. 3. Bacteroides melaninogenicus, microscopic morphology. Organisms are coccobacillary.

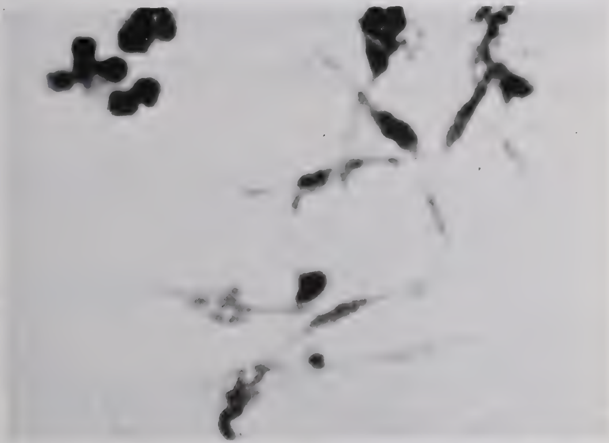

FIG. 4. Fusobacterium mortiferum, microscopic morphology F. varium and F. necrophorum have a similar appearance. Note striking pleomorphism, with long filaments (some of which contain swellings) and large round bodies. There is irregularity of staining also. (From Finegold, S. M., Med. Times 96: 176–181, 1968)

choice in adults (assuming there are no contraindications to this procedure) and direct lung puncture, in children. Specimens obtained through a bronchoscope are not suitable. Specimens aspirated from patients with tracheostomies should be suitable. The above-mentioned procedures are necessary in order to bypass the normal flora of the upper respiratory tract and

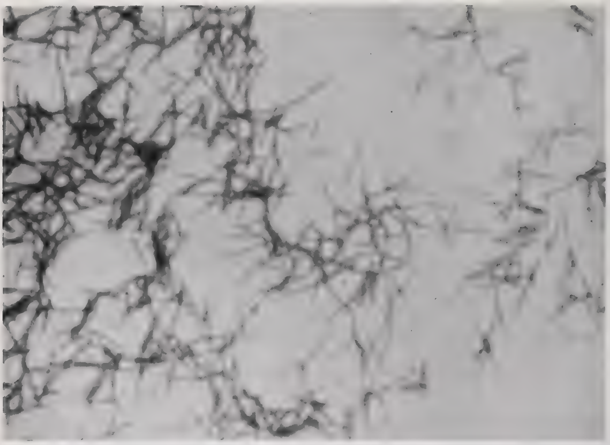

FIG. 5. Fusobacterium nucleatum, microscopic morphology. The organism is delicate with tapered ends. It takes a pale stain. (From Finegold, S. M. and Miller, L. G. in: *The Anaerobic Bacteria*, Montreal; Montreal Univ., 1968)

mouth which contain a large variety of anaerobes in great numbers. In suspected anaerobic infection of the urinary tract, voided urine cannot be used because of the presence of a variety of anaerobes as normal flora on the urethral mucosa. Accordingly, one must use suprapubic bladder aspiration to obtain urine unless there is a concurrent bacteremia or a more reliable specimen source such as a nephrostomy tube available.

Material from abscesses is best obtained by needle and syringe aspiration of the closed abscess. The vagina also has an indigenous population of anaerobes which must be avoided in taking uterine cultures. Careful positioning of the patient and good exposure with a vaginal speculum, along with decontamination of the cervical os and vagina with an iodophor, are all important considerations. Then culdocentesis may be used to obtain material from the pelvic area or a small plastic tubing can be inserted through the cervical os into the endometrial cavity and a syringe attached for aspiration of material.

Specimens which are normally sterile such as spinal fluid, blood, and joint fluid may be collected in the usual fashion after thorough skin decontamination. In general, material for anaerobic culture is best obtained using a needle and syringe. Air and gas are then expelled from the syringe and needle. The use of swabs is a poor alternative for a number of reasons, including excessive exposure of the specimen to the deleterious effects of oxygen and drying.

The second major consideration relates to the possibility of anaerobes dying as a result of exposure to oxygen during the interval between obtaining the specimen and culturing it in the laboratory. Anaerobic syringe techniques, such as are used in studies of blood gases, may prove very useful for transport (see Fig. 6). If a sterile stopper is not available, one may use a non-sterile stopper, as from a Vacutainer tube. The laboratory should then be instructed to put a sterile needle on in place of the original one prior to setting up the specimen for culture. With the syringe technique, it is important that the specimen be delivered to the laboratory and set up in culture within 20 to 30 minutes. When longer periods are required for transport, a gassed-out tube with a butyl rubber stopper, as shown in Figure 7, is very convenient to use (13). This tube has been gassed out with oxygen-free gas and incorporates an indicator so that one may be certain that the atmosphere is anaerobic. The indicator may be incorporated either in agar or in a fluid diluent (Fig. 8). We use resazurin, a very sensitive oxidation-reduction indicator, which turns pink on exposure to air. Once the specimen has been injected into the tube, even a two-hour delay between collection and setting up the specimen in culture is satisfactory. One can hold specimens in such tubes for extended periods (over 24 hours, if necessary) if the tube is refrigerated to prevent overgrowth of aerobic and facultative forms. If it is absolutely necessary to use a swab to obtain a specimen, a setup such as is pictured in Fig. 9 is recommended. The swab itself is put up in an anaerobic atmosphere. After the specimen has been collected, the swab is placed into the second tube which contains a deep column of semisolid transport medium such as Cary and Blair medium.

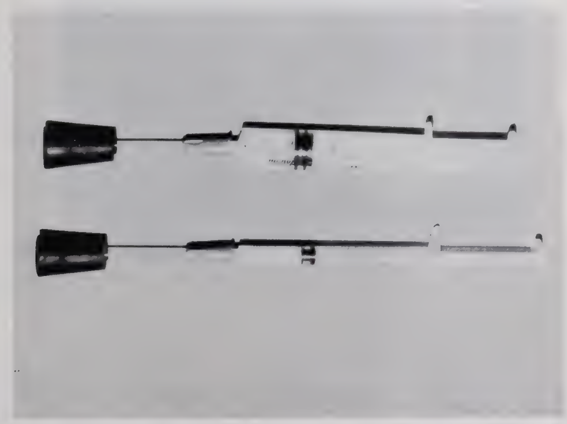

Fig. 6. Syringe transport technique. Useful only for short periods of transport (20 minutes or less). All air has been expelled from the syringes and needles.

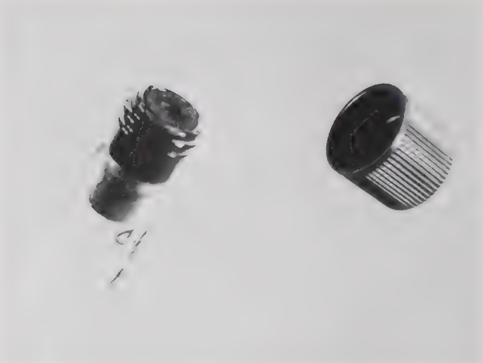

FIG. 7. Anaerobic transport tube. The atmosphere in this tube is oxygen-free gas. It is stoppered with a recessed butyl rubber stopper.

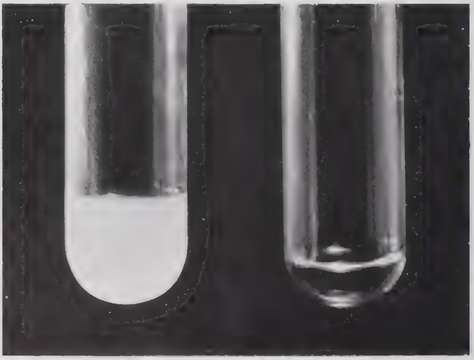

FIG. 8. Anaerobic transport tube. An oxidation-reduction indicator is incorporated in a small amount of agar or liquid in the bottom of the tube.

Anaerobic Culture Techniques

Two recent studies (14, 15) have shown that, when clinical specimens are properly collected and transported, recovery of clinically significant anaerobes is as good with anaerobic jars (including the simple GasPak jar) as with other more complex methods. Laboratories which have anaerobic chambers or the equipment necessary for the Hungate technique or modifications of it may still

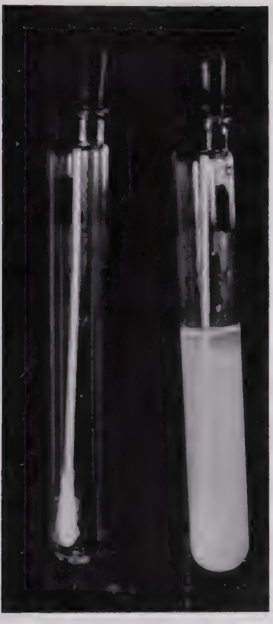

FIG. 9. Anaerobic swab transport setup. The swab and the transport medium (semisolid Cary and Blair medium) are each put up in anaerobic tubes.

find it convenient to use these procedures at various points in the culture technique. If specimen transport has been less than optimal, so that organisms in the specimen are reduced in numbers and perhaps are in poor condition, use of the chamber or Hungate techniques may result in a better yield than would be possible with anaerobic jar techniques.

A number of different types of anaerobic jars are available. Three of the more popular jars are shown in Figure 10. The use of a gas mixture containing hydrogen is followed by catalytic conversion of the hydrogen and oxygen in the jar to water, leading to anaerobic conditions. Carbon dioxide is always used as part of the atmosphere since many anaerobes either require it or grow better with it in the atmosphere. A "cold" catalyst, composed of palladium-coated alumina pellets held in a wire mesh, is the preferred type. This type of catalyst should be reactivated after each use by heating the container of pellets to 160°C in a drying oven for 1-½ to 2 hours. It is convenient to have a few extra baskets of catalyst on hand for this purpose. Reactivated catalyst can be

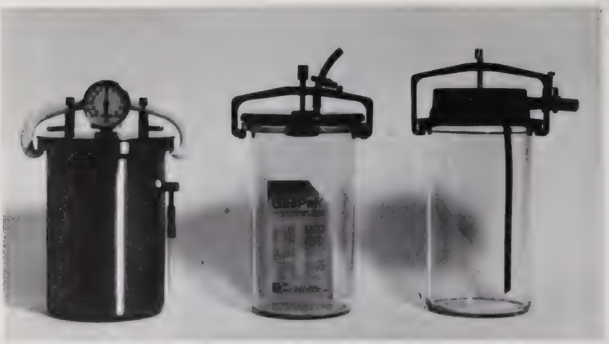

FIG. 10. Anaerobic jars in common use. Torbal jar on left, GasPak in center, and Brewer jar on right. The Torbal and GasPak jars use a "cold" catalyst. The Brewer jar is no longer available.

stored for extended periods in a dry place. With the GasPak technique, a commercially available envelope generates hydrogen and carbon dioxide after addition of water. An alternative procedure utilizes evacuation and replacement. Air is removed from the sealed jar containing the culture plates by drawing a vacuum of 25 inches of Hg; then, the jars are filled with an oxygen-free gas such as nitrogen. This process is repeated 5 to 7 times. The final fill of the jar is made with a gas mixture containing 80% nitrogen, 10% hydrogen and 10% carbon dioxide. This procedure achieves anaerobiosis more quickly than does catalytic conversion without any evacuation and replacement. This may be an advantage in the case of clinical specimens which have not been transported properly. With the GasPak system, it is important to make the jar anaerobic as soon as the inoculated plates have been put in. This can be done by activating the envelope, but if one does not have a full jar one could gas the jar with flowing oxygen-free gas (16) until additional specimens allow one to set up a full jar with the GasPak envelope.

Processing and Examination of Clinical Specimens

It is not possible to give explicit details in this paper. The interested reader is referred to other publications for such details (17, 18).

The direct examination of the clinical specimen, by Gram stain and preferably also by darkfield examination, cannot be stressed too much. These examinations help ensure that the specimen has been properly obtained. They may give immediate presumptive evidence of the presence of anaerobes on the basis of unique morphology. They may suggest the desirability of using special media in the culture procedure which would enhance the recovery of certain organisms suspected because of their distinctive morphology on direct smear (i.e., Actinomyces). The desirability of utilizing certain selective media may also be suggested. Rough quantitation of the various elements in a mixed infection is also possible from the direct smear. The Gram stain is useful in suggesting appropriate therapy prior to the availability of culture results. Finally, it is an important quality control procedure. One should expect to recover, on culture, all of the various types of organisms seen on direct smear.

The most important basic medium is the blood agar plate. Brucella agar base yields much better growth of anaerobes than the usual heart infusion base. Vitamin K_1 is incorporated in a final concentration of 10 μg/ml. A variety of selective media is available. The one which is probably most useful is kanamycin-vancomycin laked blood agar. This incorporates 75 μg/ml base activity of kanamycin and 7.5 μg/ml vancomycin in Brucella blood agar plates with lysed or laked blood rather than whole blood. Growth on this medium is presumptive evidence of an obligate anaerobe because facultative bacteria seldom grow on it. It is most selective for Bacteroides, particularly Bacteroides fragilis and Bacteroides melaninogenicus. A liquid medium may also be used, usually as a backup medium. Thioglycollate with glucose and without indicator, enriched with hemin 5μg/ml, peptic digest of sheep blood (5%), sodium bicarbonate 1 mg/ml and vitamin K_1 0.1 μg/ml, is a good medium for this purpose. Additional media for special purposes are noted in the references cited above.

All media used should be fresh. We find that most commercial plate media are not as satisfactory for growth of anaerobes as media made in the laboratory.

Plates are incubated in jars for 48 hours prior to initial examination and then are re-incubated for one week or longer before making a final examination. Under emergency conditions, plates can be examined sooner. If this can be anticipated, it is desirable to set up duplicate cultures so that one set is not opened before 48 hours. Plates should be examined with the aid of both a hand lens and a stereoscopic microscope. Each colony type should be described and picked for Gram stain and further identification. Each colony type should be streaked to blood agar for purity and plates incubated anaerobically. Pure cultures should be tested for their ability to grow in 5 to 10% CO_2 and in air.

Identification of Anaerobes

One may question whether definitive identification of anaerobes is necessary for the clinical laboratory. For most clinical purposes, it is not necessary to have final identification for management of the patient's infection. It is my feeling that all laboratories should be able to identify Bacteroides fragilis and Clostridium perfringens relatively rapidly. Bacteroides fragilis is the most common anaerobe encountered, occurring in 60% of all anaerobic infections. It is also important in that it is the most resistant of all anaerobes to antimicrobial agents. Clostridium perfringens, a much less commonly encountered pathogen, nevertheless is important inasmuch as it may produce very devastating illness. Beyond this, the immediate needs of the patient may be served adequately by informing the clinician as to the general nature of the organism (anaerobic Gram-negative bacillus other than B. fragilis, anaerobic streptococcus, anaerobic Gram-positive nonspore-forming catalase-negative bacillus, etc.) and, at times, to provide antimicrobial susceptibility testing of the isolate.

Despite what has been said above, it is desirable for as many laboratories as possible to provide reliable identification of anaerobic isolates. There are a number of reasons for this. First of all, the broad general categories of bacteria may be very difficult to define in the case of anaerobes. For example, Gram-positive anaerobes often destain readily and may appear Gram-negative. Certain bacilli, such as Bacteroides melaninogenicus, are quite small and may appear to be cocci. It may be very difficult to demonstrate the presence of spores. Definitive identification of an isolate may provide valuable information to the clinician as to a likely portal of entry or source in the case of bacteremia of uncertain origin. The identity of a given isolate may influence the patient's prognosis. For example, the mortality in subacute bacterial endocarditis due to Bacteroides fragilis is distinctly higher than in endocarditis caused by other Gram-negative anaerobic bacilli or anaerobic cocci. The strong association between Clostridium septicum infection and malignancy underlines the desirability of knowing the identity of this isolate when it is encountered in infection. In certain cases, accurate identification of species may permit differentiation between relapse of an old infection and a new infection in the same patient. Finally, of course, careful bacteriologic (and clinical) study of anaerobic infection will permit us to gain much needed information concerning involvement of various anaerobes in different infectious processes, the prognosis associated with each, etc.

Our laboratory has found it useful to utilize a number of simple tests and observations for preliminary grouping of anaerobic bacteria. Included are observations concerning colony morphology, pigment production, hemolysis, pitting of the agar, fluorescence under ultraviolet light and Gram reaction and microscopic morphology. All of these observations, as well as the spot indole test (19) can be done using colonies on blood agar plates. Young cultures in enriched thioglycollate broth should be examined for Gram reaction, morphology and motility. Colony morphology in broth may also be of interest at times. Figure 11 shows the growth of Fusobacterium necrophorum in balls in a blood culture broth. Figure 12 shows the distinctive appearance of Clostridium perfringens on a blood agar plate. The double zone of hemolysis is an important clue suggesting that the organism may be C. perfringens.

From the enriched thioglycollate broth, subcultures may be made to blood agar plates for antibiotic disc identification and to egg yolk-Nagler plates. The antibiotic disc identification procedure (20) utilizes up to 6 antibiotic impregnated discs, most of which are not the standard concentrations used for the usual susceptibility testing. Details on the procedure for performance of these tests are given in References 17, 18 and 20. The egg yolk-Nagler plate is prepared by flooding half of a plate of egg yolk agar with C. perfringens type A antitoxin. A loopful of growth from the thioglycollate medium is then streaked across both halves of the plate. After 24 to 48 hours of anaerobic incubation,

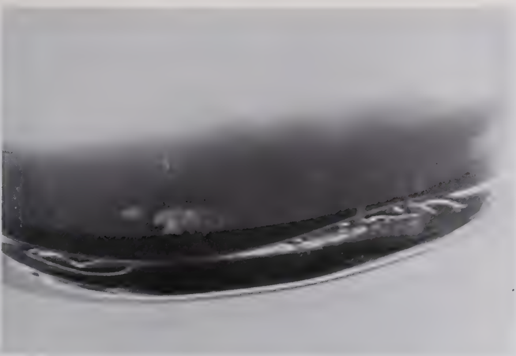

FIG. 11. Fusobacterium necrophorum colonies in blood culture bottle. Note growth in balls.

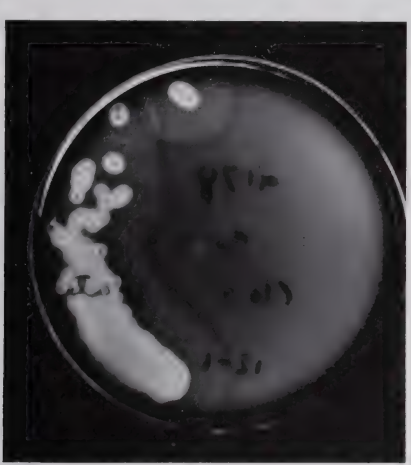

FIG. 12. Clostridium perfringens colonies on blood agar plate. Note double zone of hemolysis—inner zone of intense beta hemolysis and less intense outer zone.

plates are observed for the lipase and lecithinase reactions and the Nagler reaction (inhibition of the lecithinase reaction by the antitoxin). Figure 13 illustrates both the lipase and lecithinase reactions on egg yolk agar with Clostridium novyi type A. A positive Nagler reaction with C. perfringens is

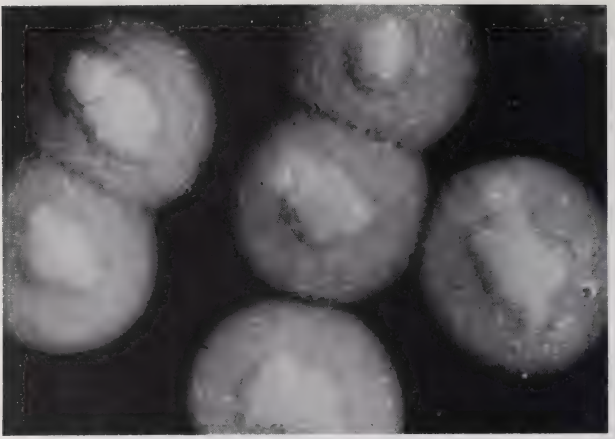

FIG. 13. Clostridium novyi type A colonies on egg yolk agar. The organism produces both lecithinase (zone of opacification of medium about colony) and lipase (sheen on surface of colony).

shown in Figure 14. An additional simple test which is useful for characterizing anaerobes is the bile test (17, 21).

Bacteroides fragilis can be identified by the fact that it is an obligately anaerobic Gram-negative bacillus with rounded ends that is resistant (zone of inhibition less than 10 mm) to the discs containing colistin, kanamycin, penicillin and vancomycin in the special disc identification procedure referred to above. It is sensitive to the erythromycin and rifampin discs. It is not inhibited, and is usually stimulated, by 20% bile. A simple bile-kanamycin test for identification of Bacteroides fragilis has been described by Vargo et al (22). Subspeciation of Bacteroides fragilis can be carried out by the addition of three carbohydrate fermentation tests (mannitol, rhamnose and trehalose) and indole.

Clostridium perfringens is identified rather readily. Its characteristic microscopic morphology, the double zone of hemolysis which may be seen, and the positive Nagler reaction all suggest this particular organism. However, there are 3 other alpha-toxin producers which may give a positive Nagler reaction which must be distinguished from C. perfringens. The lack of motility of C. perfringens distinguishes it from C. bifermentans and C. sordellii, while its ability to hydrolyse gelatin distinguishes it from C. barati.

The API (Analytab, Inc.) strips for anaerobes provide carbohydrate and other simple tests in a convenient and relatively inexpensive form. These may be useful aids for identification of rapid growing nonfastidious anaerobes. Ultimately, identification of a number of anaerobes does require analysis of end-products of metabolism by gas chromatography. This will not be practical for all laboratories but should be available at least in teaching hospitals.

Susceptibility Testing; Therapy of Anaerobic Infections

Disc diffusion antibiotic susceptibility tests have not been standardized for all anaerobic bacteria. The Kirby-Bauer standards for disc susceptibility of aerobic or facultative bacteria must *not* be used with anaerobic isolates. The procedure for the disc agar diffusion test for rapidly growing anaerobes and tentative standards for interpretation of susceptibility may be found elsewhere (18).

Tube dilution and agar dilution susceptibility tests are also not standardized with regard to the anaerobes. Nevertheless, these tests may be useful for guiding therapy in selected cases. Details on the techniques which may be utilized are given elsewhere (18).

The usual patterns of susceptibility of various anaerobic bacteria to the drugs which are most useful for anaerobic infections are noted in Table 4.

Obviously, other aspects of therapy may be very important as well. In general, drainage of abscesses and surgical debridement of wounds are extremely important for assuring a successful outcome and may be more important than antimicrobial therapy. In many situations, both play an impor-

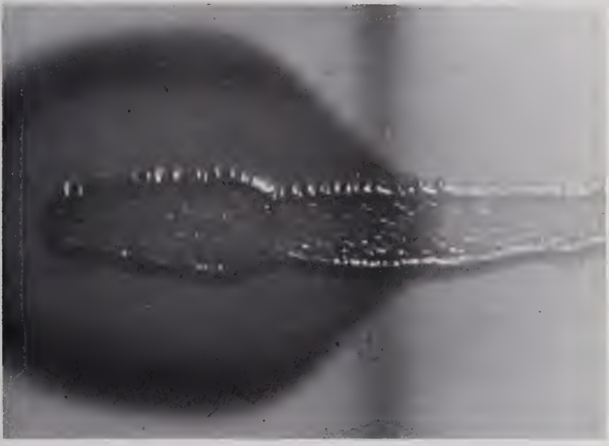

FIG. 14. Positive Nagler reaction, C. perfringens, Organism is streaked across egg yolk agar plate, the right half of which has previously been swabbed with antitoxin to the alpha toxin. On the left half, one sees a lecithinase reaction. This reaction is completely blocked on the right half by the antitoxin, the diffusion of which accounts for the tapered shape of the lecithinase reaction near the center of the plate.

TABLE 4

Susceptibility of Anaerobes to Antimicrobial Agents

	Microaerophilic and Anaerobic Cocci	Bacteroides fragilis	Bacteroides melaninogenicus	Fusobacterium varium	Other Fusobacterium Species	Eubacterium and Actinomyces	Clostridium perfringens	Other clostridia
Penicillin G	+++ to ++++	+	+++[b]	+++[a]	+++[a]	++++	++++[a]	+++
Lincomycin	+++	+ to ++	+++	++	+++	++ to +++	++ to +++	+
Clindamycin	++ to +++	+++	+++	++	+++	+++[b]	+++[b]	++
Metronidazole	++	+++	+++	+++	+++	+ to ++	+++	+++
Chloramphenicol	+++	+++	+++	+++	+++	+++	+++[a]	+++[b]
Tetracycline	++	+ to ++	++ to +++	++	++ to +++	++ to +++	++	++
Erythromycin	++ to +++	+ to ++	+++	+	+	+++	+ to ++	++ to +++[c]
Vancomycin	++ to +++	+	+	+	+	++ to +++	+++	++ to +++[a]

++++ = Drug of choice; +++ = Good activity; ++ = Moderate activity; + = Poor or inconsistent activity.

[a] A few strains are resistant.

[b] Rare strains resistant.

[c] Based on old studies (resistance may have developed subsequently).

tant role. In certain conditions other modes of therapy such as the use of antitoxin, exchange transfusion, etc. may play a role.

Common Errors in Anaerobic Bacteriology

1. Gram stain not prepared directly from clinical specimen. The Gram stain alerts one to the possible presence of organisms requiring special media or conditions of incubation. It also helps one to realize that his techniques are defective if he fails to grow out organisms seen on the smear.
2. Failure to set up anaerobic cultures promptly from clinical specimens —or to keep these under anaerobic conditions pending culture.
3. Use of fluid thioglycollate medium as the only system for growing anaerobes. A number of anaerobes will not grow in this medium, even when it is enriched, and solid media are required to separate the various organisms present in mixed culture. Errors are made at times even when anaerobes have grown in fluid thioglycollate medium. Some workers do not check this medium if growth appears on aerobic plates, assuming that the same organism is growing in both. A Gram stain of the broth will often alert one to the additional presence of anaerobes.
4. Use of inadequate commercial media. Failure to use fresh media.
5. Failure to use supplements in media. Vitamin K_1 is required by some strains of B. melaninogenicus.
6. Failure to use selective media. Some anaerobes may be overgrown by facultative anaerobes and overlooked if selective media are not used.
7. Failure to use a good anaerobic jar. Brewer, McIntosh-Fildes. Torbal or Baird-Tatlock, and GasPak jars have been found to perform well.
8. Failure to check jars carefully for leaks after they are set up.

9. Catalysts not in good working order when using hydrogen in jars.
10. Using too few flushes when not using hydrogen in jars.
11. Using toxic gas in displacement procedure.
12. Failure to include 5 to 10% CO_2 in atmosphere in jars. Carbon dioxide is essential for some anaerobes.
13. Failure to hold cultures for extended periods. Occasionally, fastidious organisms present in small numbers may require 2 to 3 weeks to grow.
14. Failure to minimize air exposure during processing.
15. Failure to use redox indicator or known fastidious anaerobe in jar.
16. Inaccurate identification and speciation.
17. Use of disc susceptibility technique without standards (or use of Kirby-Bauer aerobic standards).
18. Failure to determine whether or not organism is a true anaerobe.

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Venereal Disease—Clinical and Laboratory Diagnoses

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It was the momentous discoveries of Treponema pallidum by Schaudinn and Hoffman (1905), of the serologic reaction by Wassermann (1906), and of the organic arsenicals by Ehrlich and Hata (1910) that constituted, as Stokes et al stated, "a triumph of constructive thinking that within one decade raised empirical medicine . . . to the dignity of an exact science" (1). Since that time, our knowledge of venereal disease has expanded, our skill for detection has increased, our therapeutic weapons have multiplied. Outstanding diagnostic contributions have been Kolmer's (2) modification of the Wassermann reaction, Kahn's (3) introduction of a quantitative precipitation reaction for syphilis, the purification of cardiolipin by Pangborn (4) thereby providing consistency of serologic results and the development of the VDRL serologic test as described by Harris and his colleagues (5). Specific serologic procedures for treponemal antibodies were first introduced by Nelson and Mayer (6) and their famous Treponema Pallidum Immobilization (TPI) test which was followed by the Fluorescent Treponemal Antibody (FTA) test of Deacon and associates (7). Research in gonorrhea brought forth the introduction of highly selective media such as Thayer-Martin's (8) and New York City (NYC) (9) for the isolation of pathogenic Neisserian organisms. Other notable disoveries such as the colonial biotypes by Kellogg et al (10) and of pili by Swanson and collaborators (11) hopefully may lead to the development of highly sensitive and specific serologic tests for gonorrhea.

Yet, in spite of these advances for diagnosing gonorrhea and syphilis, the incidences of these diseases have reached epidemic proportions. Gonorrhea, the most frequently reported communicable disease (12) (Fig. 1), continued to increase during 1974 and has more than doubled in six years (Fig. 2). In fiscal year (FY) 1974 there were 874,161 reported cases of gonorrhea, 64,480 cases more or 8.0 percent higher than the previous year. Because the majority of women and a lesser proportion of men with gonorrhea lack clinical signs or symptoms, many cases remain undetected and untreated. Since many cases of gonorrhea are not detected and many which are treated are not reported, the true incidence of the disease is unknown. It is estimated by the Public Health Service that new cases of gonorrhea in the United States are now occurring at a rate of 2,700,000 annually.

Syphilis is not trailing far behind. It is now the third most frequently reported communicable disease (12) (Fig. 1). Even though reported cases of pri-

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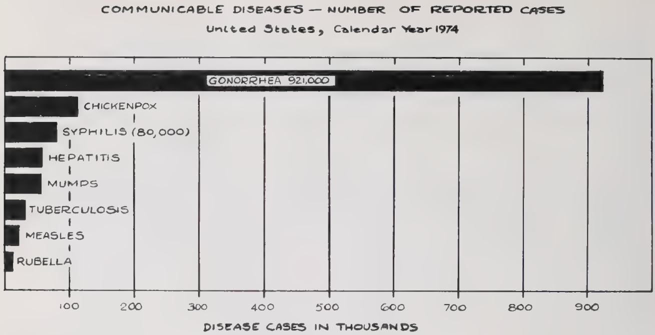

SOURCE. CDC
MORBIDITY & MORTALITY

FIG. 1. Number of reported cases of communicable diseases in the United States for calendar year 1974.

mary and secondary syphilis decreased for the first time since 1969 (Fig. 3) from 25,080 reported cases for FY1973 down to 24,728 reported cases for FY1974 or 1.4 percent less, reported early latent syphilis cases have been increasing since FY1968. This indicates that a large number of syphilis cases are not being detected in the most infectious stages—the primary and secondary. As with gonorrhea, the reported incidence of infectious syphilis represents only a small portion of the number of cases actually occurring.

The three minor veneral diseases; namely, chancroid, granuloma inguinale, and lymphogranuloma venereum, although serious in their manifestions, are rare in the United States. These three diseases account for less than 0.3 percent of the national reported incidence of venereal disease in the United States.

There are other diseases of microbiologic etiology that are sexually transmitted. These include nonspecific or nonspecified genital infection, trichomoniasis, candidiasis, genital herpes, condylomata acuminata, molluscum contagiosum, pediculosis pubis, scabies and others not yet identified. Although nonspecified genital infection may be of greater magnitude in other parts of the world (13), its incidence in the United States is not known for it is not reportable. Perhaps as Willcox states "Other sexually transmitted organisms are waiting in the wings, as it were, seeking a role" (14) possibly to take over if and when gonorrhea and syphilis are brought under control.

Physicians and laboratory workers recognize the enormous problem gonorrhea and syphilis represent in the United States. It is, therefore, pertinent to review the clinical aspects and the accepted methods of laboratory diagnosis for these diseases.

Gonorrhea

Gonorrhea is almost exclusively transmitted by sexual contact. It is commonly known in the vernacular as "clap," "strain," "the dose," "the drip," "GC" or "the whites." The etiologic agent of gonorrhea is the bacterium Neisseria gonorrhoeae commonly called the gonococcus. Characteristically in Gram-stained specimens of exudate from an acute infection the gonococci appear as Gram-negative, bean-shaped diplococci with flattened or concave

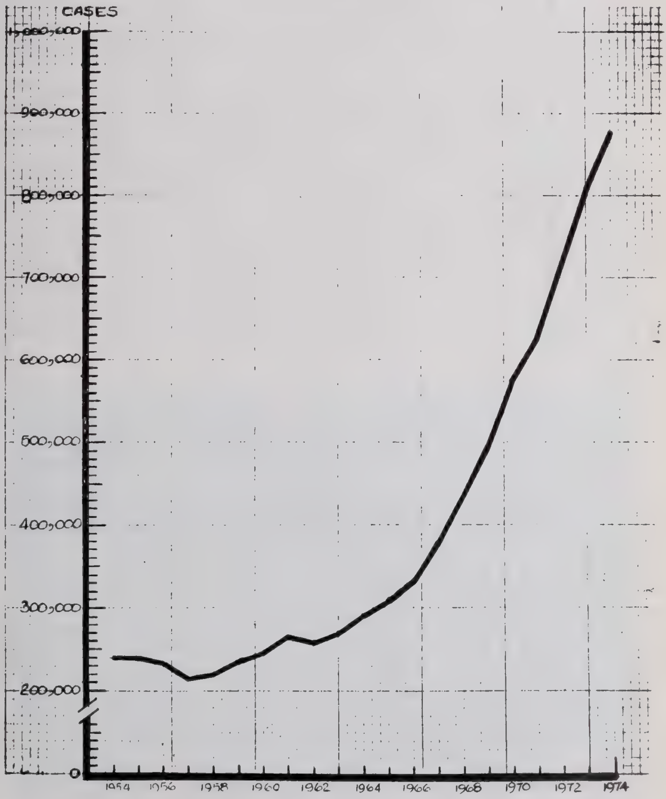

FIG. 2. Reported cases of gonorrhea in the United States for the fiscal years 1954 through 1974.

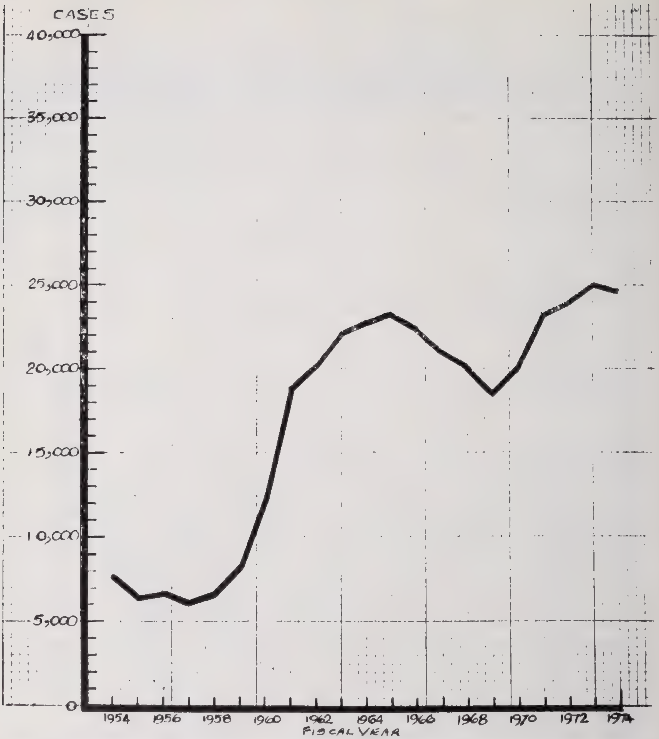

FIG. 3. Reported cases of primary and secondary syphilis in the United States for the fiscal years 1954 through 1974.

inner surfaces within a small proportion of polymorphonuclear leucocytes (Fig. 4). Extracellular gonococci also occur. Their size varies from 0.6 to 1.0 micron in diameter. When exposed to antibiotics, the gonococci progressively enlarge and decrease in stainability until none can be detected.

Gonococci are fastidious in their growth requirement. They do not grow on nutrient agar without enrichment with lysed whole blood and/or ascitic fluid and the required growth supplements. Optimal growth is obtained in a moist atmosphere under increased carbon dioxide tension (2–10 per cent) at 35° to 36° degrees C.

Gonococci have been divided into four types (Types 1 to 4) on the basis of colonial morphology (10). Electron micrographs have shown that Types 1 and

2 possess hair-like appendages called pili or fimbriae, whereas Types 3 and 4 do not (11). These colonial variations have been associated with pathogenicity (or virulence), as only Types 1 and 2 produce gonorrheal urethritis in male volunteers (15); Types 3 and 4 are avirulent.

The gonococci rarely ever invade or multiply in tissue covered with squamous epithelium. However, columnar and transitional epithelia offer no resistance. Due to this fact and the primary modes of exposure the most common sites of primary infection are the urethra in the male and the cervix and urethra in the female.

Clinical Findings: In the male, the disease usually starts as an acute anterior urethritis. Signs and symptoms usually develop two to seven days after exposure, although the incubation period may on occasion be as long as a month. It has been reported that as high as 10 percent of males with gonococcal urethritis may be completely asymptomatic (16). Although this percentage is debatable, the existence of asymptomatic gonococcal infection of the urethra must be recognized as a reality.

In symptomatic urethritis initially there is burning on urination which may be limited to the external urethral meatus. This burning sensation gradually diffuses upward to include the entire urethra. With the onset of dysuria on the same day or on the day after, a urethral discharge appears which may be scanty and serous but rapidly becomes profuse, thick, yellowish in color, and purulent (Fig. 5). Additionally there may be frequency of urination. Constitutional symptoms, if present, are mild. Because of these visible signs and symptoms, the male with acute gonococcal urethritis usually seeks medical

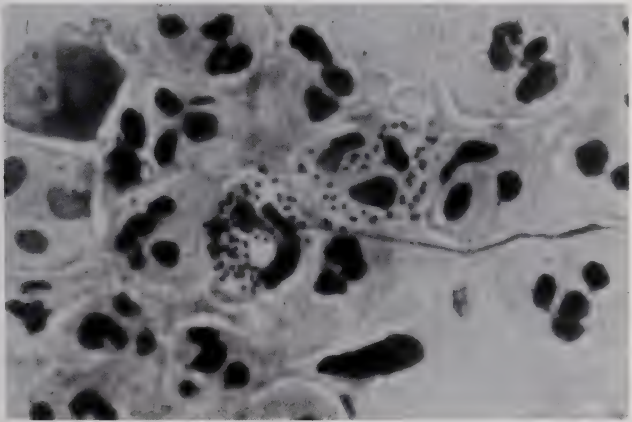

FIG. 4. Neisseria gonorrhoeae. Gram-stained preparation from urethral exudate showing Gram-negative intracellular diplococci within polymorphonuclear leucocytes.

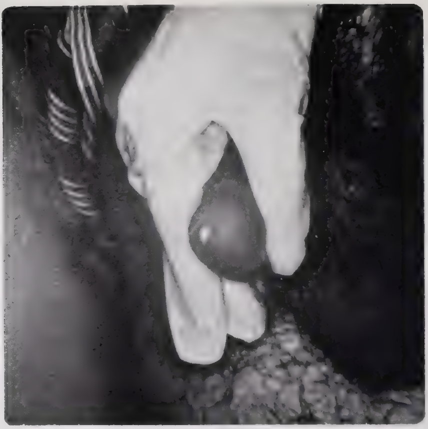

Fig. 5. Gonococcal urethritis with profuse, purulent urethral discharge.

attention. Adequate treatment of this stage of gonorrhea will result in cure and prevent the genitourinary complications.

However, if the anterior urethritis is not treated, within 10 to 14 days the infection will spread to the posterior urethra. With involvement at this site, the dysuria frequently becomes more severe. The urethral discharge may remain purulent or become pyosanguinous. Constitutional symptoms are more likely to occur. These include pyrexia, cephalalgia, tachycardia and malaise. With continued spread of the gonococci, cowperitis and seminal vesiculitis may ensue although distinctive symptoms may not appear. Extension of the organisms along the vas deferens to the epididymis may result in a gonococcal epididymitis. Generally the infection at this site is unilateral and results in severe pain, swelling and tenderness in the scrotum on the infected side. Fibrous scarring may obliterate the seminiferous tubules and in cases of bilateral involvement, the result may be complete, irreversible sterility.

Infection of the prostate gland may result in an acute gonococcal prostatitis. An abscess may form with symptoms that may include severe rectal pain and discomfort, painful defecation, pain in the perineum, terminal dysuria and/or rectal tenesmus. Rectal examination reveals a large, tense and very tender swelling which bulges into the rectum and rises into the pelvis.

Unlike the typically symptomatic male, the infected female with acute gonorrhea is a diagnostic challenge. Approximately 80 percent of females with acute gonorrhea may be completely asymptomatic (17). The majority of these females remain untreated, constitute a reservoir for spread of the disease and are candidates for the later, destructive, complications of gonorrhea.

Because of the asymptomatic nature of the disease in females, it is difficult adequately to determine the incubation period. In those who develop symptoms, the most constant presenting symptoms are dysuria and increased vaginal discharge. In addition, frequency of urination, lower abdominal discomfort, low backache or occasional discomfort in the rectal area may be present.

As stated earlier, the two most common sites of primary infection in the female are the mucosal lining of the cervix and the urethral canal. On examination one may notice swelling, redness and pus in the urethral canal. The vulva may appear swollen and inflamed and the cervix may be inflamed and bathed in a purulent discharge (Fig. 6). However, none of these signs may be evident. With or without treatment, the acute cervicitis subsides with subsequent disappearance of any erythema or gonococcal discharge. The organisms, however, take up residence in the endocervical glands.

Complications from genital gonorrhea can in some cases appear early in the infection. In most cases, however, they result from prolonged, silent infection either undiagnosed or inadequately treated. Bartholin's glands may become involved with subsequent abscess formation if the single duct is occluded. The bladder may be infected to a mild degree, causing urinary frequency. The infection may spread to the fallopian tubes with subsequent salpingitis. Symptoms may be severe enough to warrant a diagnosis of peritonitis. More often there is a history of recurrent bouts of abdominal pain, low grade fever, low back pain, dyspareunia and postmenstrual pain. Chronic inflammation of

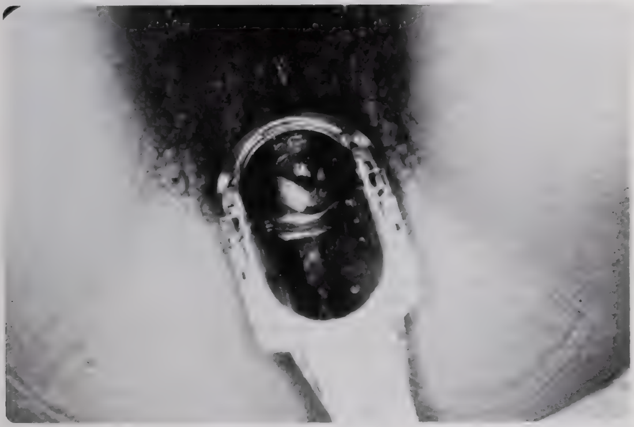

FIG. 6. Gonococcal cervicitis with purulent cervical discharge and exocervicitis. Courtesy of Dr. N. J. Fiumara.

the tubes may lead to adhesions, loss of patency with resulting infertility, pyosalpinx, hydrosalpinx, ovarian abscess and/or peritonitis.

In both males and females, other sites that may become infected primarily or secondarily are the rectum, nasopharynx and conjunctiva. Gonococcal proctitis in the male is nearly always due to rectal intercourse. In the preantibiotic days, rectal gonorrhea also occurred through the opening of a prostatic abscess or abscess of Cowper's gland into the anorectal lumen. Studies by Schroeter and Reynolds show that 40 to 60 percent of the cases of gonorrhea involving the genitals in females have rectal involvement (18). Infection in females may be due to autoinoculation of the rectum from vaginal secretions or to rectal intercourse. In most cases, rectal gonorrhea is asymptomatic. However, there may be anal pruritis, burning pain in the rectum, tenesmus and pain on defecation. Pus may be found in the stools. Proctoscopic examination may reveal a red and edematous rectal wall, ischio-rectal abscess, perineal abscess or anal fissure.

Pharyngeal gonorrhea is more common than is generally supposed. Studies by Wiesner et al (19) have shown that among patients with gonorrhea, the frequency of pharyngeal gonococcal infection is 10 percent among females and 21 percent among homosexual males. Most patients with pharyngeal gonorrhea are asymptomatic; those with symptoms may present with pharyngitis or tonsillitis with accompanying cervical lymphadenopathy.

Gonococcal conjunctivitis is rare in adults. It results from direct inoculation of the gonococcus onto the conjunctiva either manually or in the case of newborns from the infected mother's birth canal. The incubation period is one to three days. The conjunctiva is intensely red and swollen and emits a profuse purulent discharge (Figs. 7A, 7B). If the infection is recognized and treated properly full recovery is the rule, but in neglected cases corneal ulceration and panophthalmitis may result in blindness.

Intrauterine gonococcal infection which Handsfield and his colleagues call the "gonococcal amniotic infection syndrome" may be a common form of neonatal gonococcal infection. Gonococci were isolated from the orogastric aspirates of 14 newborns by Handsfield et al (20). Those infants harboring the gonococci had an increased morbidity when compared with infants free of N. gonorrhoeae. There was a greater incidence of prematurity, inflammation of the fetal adnexa, histologic evidence of chorioamnionitis, prolonged rupture of the fetal membranes, a greater incidence of sepsis in the infants and associated maternal peripartum fever. Newborns presenting the above signs should be considered for possible oropharyngeal gonococcal infection.

Initially gonorrhea is localized. However, gonococcemia may result when the organisms are blood-borne from their local or regional foci. This systemic spread may set up secondary foci of infection in nearly any organ system. Gonococcal arthritis is the most common systemic or disseminated complication of gonorrhea. Clinically it occurs in two stages—an early or bacteremic stage followed by a late or septic joint stage. The bacteremic stage lasts about three days and the patient usually presents with fever, chills, skin lesions and polyarthritis or tenosynovitis usually involving the medium-sized joints. The

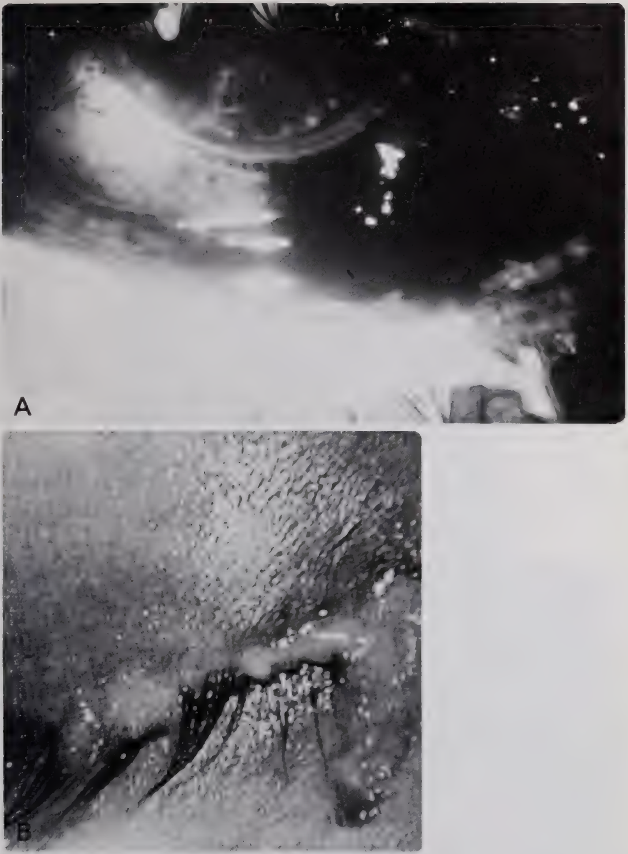

FIG. 7A. Gonococcal ophthalmia with subconjunctival hemorrhages.
FIG. 7B. Gonococcal ophthalmia showing profuse purulent discharge.

skin lesions may start as small erythematous papules or petechiae (Fig. 8) which may either disappear or progress until vesicular and pustular formation occurs. The skin lesions usually heal spontaneously within four days. Large bullae may also occur (Fig. 9). During this stage there is insufficient fluid for aspiration. The gonococcus usually can be cultured from the blood within two days after onset of symptoms rarely after five days (21). The gonococcus, at times, may be found in the skin lesions. The second stage of gonococcal arthritis, the septic joint stage, is usually characterized by a monoarticular arthritis with effusion, warmth and tenderness in the affected joint. There are no skin lesions. Blood cultures are more often negative but synovial fluid cultures more often postive.

There are other systemic complications of gonorrhea. These include endocarditis, myopericarditis, meningitis, toxic hepatitis and fulminating gonococcemia. These are rare complications and are usually coexistent with gonococcal arthritis. Patients with systemic complications usually have no symptoms at the primary site of infection.

Laboratory Diagnosis: The principle of laboratory diagnosis basically depends on proving the existence of the etiologic agent or on demonstrating results of its interaction with the human host. Diagnostically, for the present time, our armamentarium is limited to identification of Neisseria gonorrhoeae —either by direct microscopic examination and/or by cultural methods. The gonococcus can be differentiated from other bacteria morphologically by Gram's stain (or by coating with fluorescein-conjugated antigonococcal serum), by colonial morphology, by showing an oxidase-positive reaction and by fermenting only glucose (Table I).

The diagnosis of acute, uncomplicated, gonococcal urethritis in the male is not difficult to make. The diagnosis usually is based on history, characteristic clinical signs and symptoms and direct microscopic examination of slide preparations of the discharge. The discharge is collected from the penile

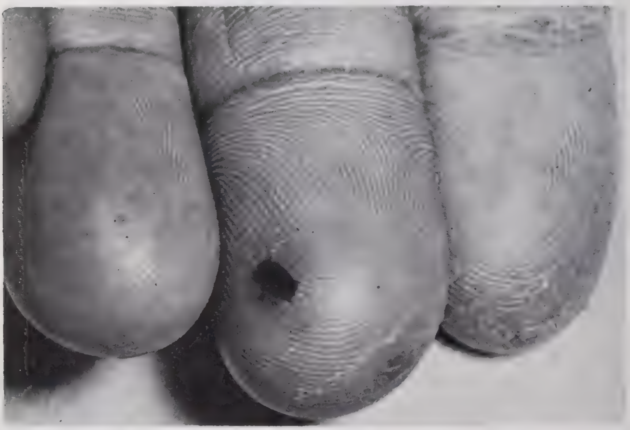

FIG. 8. Gonococcemia. Early petechia on finger. These skin lesions are usually associated with the bacteremic stage of gonococcal arthritis. Courtesy of Dr. K. K Holmes.

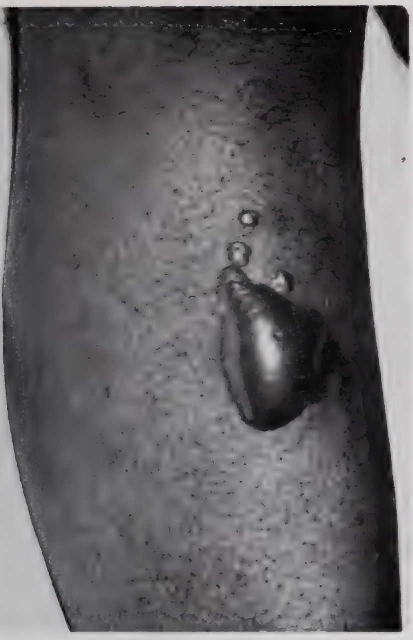

FIG. 9. Gonococcemia. Bullae on exterior tibial surface. Courtesy of Dr. K. K. Holmes.

urethra with a cotton tipped applicator stick, rolled thinly on a microscopic slide, fixed and Gram stained. The urethra may be gently stripped if there is no visible discharge. The finding of typical Gram-negative intracellular diplococci within the pus cells of the urethral discharge is all that is necessary for a laboratory diagnosis. While a positive Gram stain combined with the presence of typical signs and symptoms confirms the presence of gonorrhea in the male, a negative microscopic finding does not rule out gonococcal infection. If the Gram stain is negative in symptomatic males or where history indicates the possibility of infection in asymptomatic males, cultural evidence may be obtained by inoculating a specimen on a selective medium. The specimen is obtained from the fossa navicularis with a small wire loop after the urethra has been stripped free of any purulent discharge which may contain nonviable organisms.

A differential diagnosis of nongonococcal urethritis may be made in the presence of a positive clinical and negative cultural findings for Neisseria

TABLE I

Identification of Neisseria gonorrhoeae

1. Direct smear examination. Presence of Gram-negative, bean-shaped diplococci within polymorphonuclear leucocytes. Recommended only for male patients with acute uncomplicated urethritis.
2. Culture examination on Thayer-Martin or NYC medium. Cultures must exhibit:
   a. Typical colonial morphology
   b. Oxidase-positive colonies
   c. Gram-negative diplococci
3. Confirmation of culture examination by:
   a. Carbohydrate fermentation reaction
   b. Fluorescent-antibody technique

gonorrhoeae. Even though the etiologic agent or agents for nonspecified urethritis have not been identified (13), there are other causes of nongonococcal urethritis. Cultures for the presence of staphylococci, streptococci, candida, mycoplasma and other agents may be inoculated for identification. A wet preparation may be examined microscopically for Trichomonas vaginalis.

In the female, the diagnosis of gonorrhea is more difficult than in the male. Direct smear examination of exudates from infected sites is far too unreliable and unproductive. The main difficulty in interpreting Gram-stained smears is the presence of a mixed bacterial flora many of which may mimic gonococci and the absence or very scanty presence of intracellular diplococci (due to the asymptomatic thus chronic nature of the infection). Effete staphylococci and enterococci may stain as Gram-negative diplococci; coccobacilli (Mimeae) as well as nonpathogenic Neisseria and Veillonella may all resemble gonococci and thus induce the unwary to make false-positive laboratory diagnoses.

The best procedure is to obtain cultures from likely sites of infection. The endocervix is the site that is most often positive. Cervical specimens are obtained by inserting a bivalve vaginal speculum so as to expose the cervix. The speculum may be moistened with warm water but not with lubricants which may be toxic to the gonococcus. The area is cleansed of any cervical mucous with a cotton ball and a sterile cotton-tipped swab is inserted into the endocervical canal. The specimen obtained is inoculated on selective media by rolling the swab and cross streaking. Urethral specimens are obtained by stripping the urethra the same way as with males.

Studies have shown that culturing the anal canal in addition to the cervix increases the diagnostic yield by 3 to 7 percent (22, 23). For maximum sensitivity in detecting female gonorrhea, a culture specimen should be obtained from the anal canal. Rectal cultures are also indicated for homosexual males. Rectal specimens are obtained by inserting a sterile swab approximately one inch into the anal canal and moving it from side to side (care should be taken not to contact feces on the swab). The use of an anoscope to secure specimens does not increase the sensitivity of the culture technique.

Specimens from these sites are inoculated onto a selective culture medium and incubated at 35° to 36°C in a carbon dioxide incubator or candle jar for 24 to 48 hours.

In addition to cervical and anorectal gonorrhea, cultural identification of N. gonorrhoeae is recommended for pharyngeal or conjunctival gonorrhea, gonococcemia and cutaneous metastatic gonorrhea. For pharyngeal gonorrhea, areas of inflammation in the tonsils and posterior pharynx are swabbed and inoculated onto selective media.

In the bacteremic stage of gonococcal arthritis Holmes and his colleagues (21) isolated gonococci from the blood only in broth cultures and never on plated blood cultures. They suggested that the number of viable organisms in each blood sample is probably small. Therefore, blood specimens (5 ml) should be inoculated into liquid medium and incubated at 35° to 36°C under increased carbon dioxide tension for at least 10 days.

Cultural identification of the gonococcus, once a laborious procedure, has been greatly simplified with the advent of two media. The most easily obtainable and best known of these is the Thayer-Martin (T-M) (24). On Thayer-Martin medium, typical colonies of gonococci are usually opaque, grayish-white, raised, glistening and convex (Fig. 10). The colony size may vary from 1 to 4 mm in diameter. However, experience in the New York City Department of Health indicates that another selective medium named NYC (9) gives comparable if not better results. Like Thayer-Martin, this medium contains antibiotic inhibitors which suppress the growth of undesired Gram-negative and Gram-positive bacteria and yeast organisms yet allowing the growth of pathogenic Neisseria. NYC medium has the added advantage of providing rapid, luxuriant growth of the pathogenic Neisseria in less than 24 hours, of inhibiting a large percentage of contaminating organisms (thus making it more selective) and of being translucent for rapidly identifying organisms growing on it.

Gonococci, as well as all the other species of Neisseria, certain bacteria and yeasts produce an oxidizing enzyme which can be tested for and used as a presumptive test. This enzyme, in the presence of air, acts on certain amines to produce colored compounds. This reaction is known as the oxidase test. The oxidase reagent is a 1.0 percent solution of di-(or tetra) methyl-paraphenylene diamine. Oxidase reagent is applied to the suspicious colonies and if Neisserian undergo color changes until they turn black (Fig. 11). These

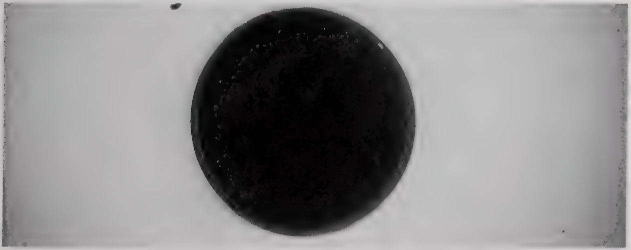

Fig. 10. Colonial morphology of Neisseria gonorrhoeae on Thayer-Martin medium.

colonies are checked microscopically for Gram-negative diplococci. If specimens inoculated on Thayer-Martin or NYC medium demonstrate typical colonial morphology, are positive for Gram-negative diplococci from the colony and exhibit a positive oxidase reaction, a presumptive diagnosis of gonorrhea can be made with confidence.

Bear in mind that the Thayer-Martin and NYC media are selective for both Neisserian pathogens—gonorrhoea and meningitidis. A confirmatory test must be performed on specimens from sites that may harbor the meningococcus and other Neisseria species such as lactamicus. Therefore, Neisseria isolated from the pharynx, blood, spinal, or joint fluid should, in addition to being oxidase-positive, also be differentiated by their ability to ferment certain sugars. Neisseria gonorrhoeae will ferment glucose but not maltose, lactose, and sucrose (Table II). The sugar fermentation reactions serve as a confirmatory test for the gonococcus.

Confirmation may also be obtained by using Fluorescent-antibody (FA) procedures (25). However, a reliable conjugate must be used. High-titer antigonococcal conjugates may be obtained by sorbing with group B meningococci to eliminate cross-reactions with meningococci and thus stain only the gonococci. Such conjugates are also sorbed with staphylococci or blocked with staphylococcal antiserum. Every lot of conjugate should be tested for reactivity with known gonococcal cells and for specificity with meningococcal and staphylococcal cells before and during use. Fluorescent antibody staining for N. gonorrhoeae should be reserved as another confirmatory procedure for organisms already isolated on Thayer-Martin or NYC media.

Because the gonococcus does not travel well and easily dies in transit, a transport medium called Transgrow (26) was developed. This medium allows the pathogenic Neisseria to grow at room temperature without incubation during transport to a central laboratory facility. Transgrow consists of a modified Thayer-Martin medium under partial carbon dioxide atmosphere in a screw-capped bottle. It eliminates the necessity for candle jars and for transferring specimens to culture plates upon arrival at the laboratory. Transgrow is of value when Thayer-Martin or NYC media are not available,

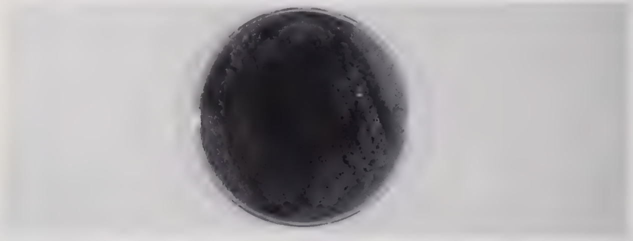

FIG. 11. Positive oxidase test showing the black color of the Neisserian colonies after applying di-methyl-para-phenylene diamine hydrochloride.

TABLE II

Sugar Fermentation Reactions of Neisseria Species

Organism	Glucose	Maltose	Sucrose	Lactose
N. gonorrhoeae	+	−		−
N. meningitidis	+	+	−	−
N. lactamicus	+	+	−	+
N. sicca	+	+	+	−

but there is a definite loss of efficiency as time in transit increases. A negative report after 72 hours or longer in transit should not be considered to exclude gonorrhea. Repeat cultures and, if possible, overnight incubation at 35° to 36°C before shipment are recommended. Although its validity under experimental conditions has been adequately established, its reliability in routine field use without overnight incubation appears quite dubious.

Another transport and culture system that is gaining popularity is the Jembec plate. It consists of a rectangular culture plate also containing modified Thayer-Martin medium. The plate incorporates a small well into which a CO_2-generating tablet is placed at the time of inoculation. The moisture in the medium breaks down the tablet into gaseous CO_2. The CO_2 environment is maintained during shipping and incubation by placing the plate into a plastic bag which is then sealed. This system is a distinct improvement over the Transgrow bottle because of its built-in CO_2-generating system, larger growth surface, easier access to the culture medium for inoculation, identification and/or subculturing procedures, and for distortion-free observation. However, like the Transgrow system, its value also depends upon prompt incubation.

Two major factors that hinder control of gonorrhea are the presence of a large number of asymptomatic carriers primarily in females and the absence of a reliable serologic test for rapid screening. Our present culture methods are not sensitive enough to diagnose all cases of asymptomatic infection. The development of a serologic test for gonorrhea of high sensitivity and specificity would be of immense value as a screening test. There have been many attempts to elaborate serologic tests for gonorrhea. Procedures for complement-fixation (27), flocculation (28), precipitin (29), microhemagglutination (30), and immunofluorescence (31) have been announced. Each test offers limited diagnostic aid for this disease, for with each one there are problems in distinguishing between infected and adequately treated individuals. Some of these procedures are still under investigation.

Syphilis

Syphilis, like, gonorrhea, is a highly infectious disease spread through intimate sexual contact with an infected person or from an infected pregnant female to her fetus. Colloquialisms such as "sif," "bad blood," and "old Joe" have been used to identify the disease. The etiologic agent of syphilis is the spirochete, Treponema pallidum, a member of a group of organisms comprising both pathogenic species, such as T. pertenue and T. carateum (agents of yaws and pinta respectively), and indigenous ones like T. refringens, T. macrodentium, T. denticola, T. orale and T. vincentii which may be found in the flora of the buccal and genitourinary tracts of humans and may cause difficulty in microscopic interpretations.

Treponema pallidum is a slender, tightly coiled helical cell with 6 to 14 rigid spirals occurring singly (Fig. 12). It may be anywhere from 6 to 20 microns in length and less than 0.2 micron in width (32). The organism has not been successfully grown in vitro but will remain motile for several days under anaerobic conditions. In vitro survival of the organism combined with serum and complement is the basis of the immobilization test for the diagnosis of syphilis (6). The organism is propagated by intratesticular inoculation of rabbits. When viewed under darkfield microscopy, T. pallidum shows characteristic motility rotating rapidly on its long axis like a corkscrew with a slight forward or backward motion. Marked directional motility is not seen with T. pallidum but frequently observed with saprophytes.

The diagnosis of syphilis involves an accurate history, clinical observations and laboratory procedures. Careful history taking is an important part of syphilis diagnosis. The history should include eliciting recollections of obscure skin rashes, injections, prolonged oral therapy, miscarriages, stillbirths, blood tests, and other information that may be suggestive of syphilis.

The necessity of a careful and complete physical examination cannot be overstressed. This examination should include the anogenital region, all cutaneous surfaces and hair, mucous membranes and lymph nodes.

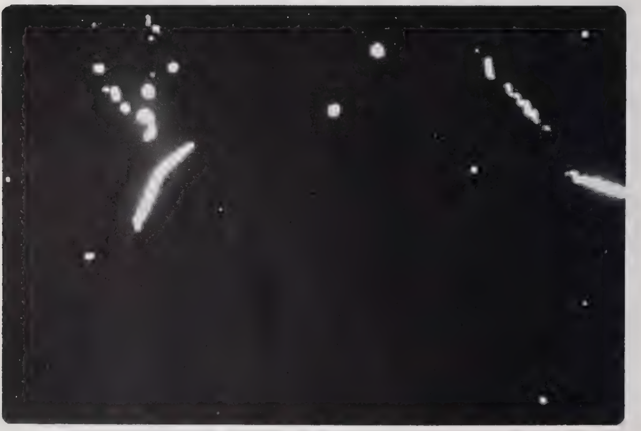

FIG. 12. Treponema pallidum. Fluorescent antibody stain.

Laboratory procedures for confirmation may include darkfield examinations, lymph node punctures, serologic tests, spinal fluid tests, histologic and roentgen-ray examinations.

The manifestations of syphilis are protean and although the disease is a continuum, its diagnosis and management are more easily understood by considering it in stages. Thus syphilis is divided into primary, secondary, early and late latent, and late (or tertiary), stages. Primary and secondary stages of the disease are infectious and early latency may be considered potentially infectious because of the possibility especially during the first two years of infection with relapsing secondaries.

Clinical Findings: The primary stage of syphilis begins with the chancre which is the first clinical evidence of syphilis. It develops at the site of the first contact with an infectious lesion and appears within 10 to 90 days after exposure although most lesions appear at just about 21 days. In most cases the lesion is single, but multiple chancres may be seen. The lesion begins as a macule which becomes papular and quickly ulcerates or erodes. The lesion is usually indurated and may have a well-defined border and a clear, granular base. Most chancres in the male occur on the coronal sulcus of the penis (Fig. 13), the frenum, the glans or the prepuce; however, they may occur in any area of the genitalia including intraurethrally. In the female the most common sites are the labia majora (Fig. 14) and minora and the fourchette; chancres may also be found on the clitoris, the cervix and the urethral orifice.

Although 95 percent of primary lesions are found on or near the genitalia (33), chancres may also occur extragenitally and may be found on the lip (Fig.

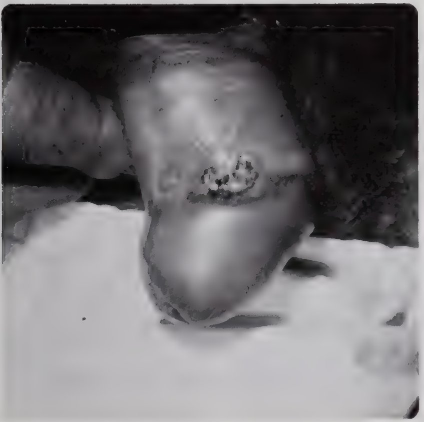

FIG. 13. Primary syphilitic chancre of coronal sulcus.

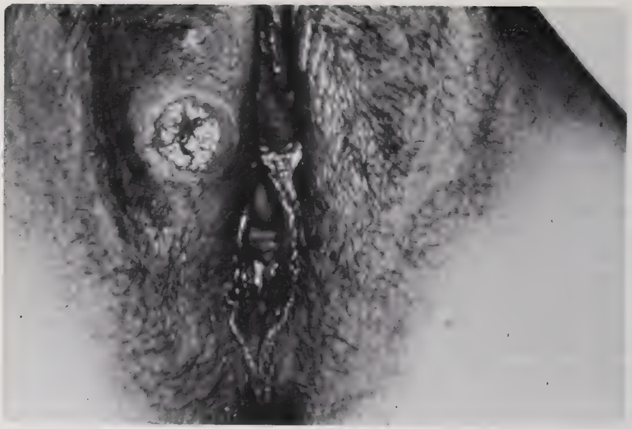

FIG. 14. Primary syphilitic chancre of labium majus.

15), the tongue, the tonsils, the anorectal area and less common sites such as the fingers and nipples. Nontender enlargement of the regional lymph nodes often accompany primary lesions. In the genital region, inguinal adenopathy is more often bilateral. Extragenital lymph node involvement is usually unilateral.

The most definitive method of diagnosing infectious syphilis is the darkfield examination. This is a microscopic examination whereby a small drop of clear serum from the lesion or aspirate from an enlarged lymph node is examined under a compound microscope equipped with a darkfield condenser. In specimens from a syphilitic lesion viable T. pallidum is visible under darkfield microscopy as a thin, motile, rotating treponeme with rigid spirals. Differentiation of T. pallidum from indigenous treponemes of the buccal cavity and genital area makes it essential to observe the organisms in the living state for observation of motility. Specimens from oral lesions are particularly difficult to interpret because of the presence of T. denticola (T. microdentium) which is indistinguishable from T. pallidum (34). When oral lesions are suspected of being syphilitic, a more accurate specimen can be obtained by aspirating a locally involved lymph node which will contain only the pathogen T. pallidum, if present. Failure to demonstrate the treponeme from a suspected lesion under darkfield microscopy may result from the patient applying a treponemicidal agent to the lesion. This may also occur when the lesion is resolving or if the specimen contains very few organisms. In such cases, aspiration of the regional lymph node and darkfield examination of the aspirate become necessary.

Ideally, the darkfield preparation should be examined immediately. If proper facilities for examination of specimens are lacking, one may collect lesion serum in 2 mm capillary tubes and after sealing the ends in paraffin wax, submit the specimens promptly to the nearest laboratory equipped to perform such examinations. This procedure is termed the "delayed darkfield examination." The chief limitation of this examination is that prolonged delay in transit may result in difficult interpretations due to complete or reduced loss of motility of the organisms. A laboratory procedure in which serum from the lesion is examined microscopically by a direct fluorescent antibody darkfield (FADF) technique to detect T. pallidum has been explored and found to be non-specific. By this method both T. pallidum as well as nonpathogenic treponemes fluoresce (35). The nonspecificity of the reaction due to the unavailability of a high titered conjugate makes this procedure undersirable for diagnostic purposes. A serologic test for syphilis should be performed when one suspects primary syphilis. The serologic test for syphilis does not become reactive until about 4 to 8 weeks after the infection is acquired. Thus it is possible that the blood test may be nonreactive for one week or more after the chancre makes its appearance. This emphasizes the importance of doing repeat darkfield examinations on suspicious lesions if found initially to be negative. Details of serodiagnosis of syphilis will be discussed later.

Whether treated or not the lesion of primary syphilis will disappear. Either before or more likely after the disappearance of this initial lesion, the lesions of the secondary stage of syphilis will make their appearance six to eight weeks

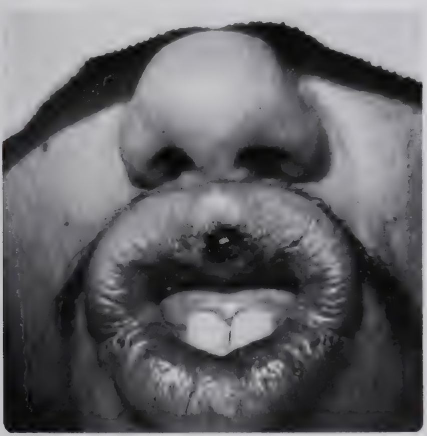

FIG. 15. Extragenital primary syphilitic chancre of upper lip located at the junction of the vermilion border and mucous membrane.

and usually within 3 months following infection. The most common manifestations of secondary syphilis involve the skin. The skin lesions which appear are usually symmetrical and nonpruritic (Fig. 16). They may be divided into four types: macular or roseolar, papular, papulosquamous and pustular. Vesicular lesions almost never occur in acquired syphilis.

The roselar lesions may appear as a slight erythema and may last a few days only. The most common type of skin lesion in secondary syphilis is the papular type and may involve the extremities, trunk, genitalia, palms (Fig. 17) and soles (Fig. 18). Follicular papules occur in the scalp and are accompanied by loss of hair. Loss of the eyelashes and the lateral third of the eyebrows may occur. Annular lesions may occur on the face, hands and genitalia. Moist, flat-topped papules, called condylomata lata, may occur in the anogenital (Fig. 19) and in other intertriginous areas such as the axilla, under the breasts and between the toes. Papular lesions may eventually show scaling and become papulosquamous in type. Pustules may form on the face and appear as acne.

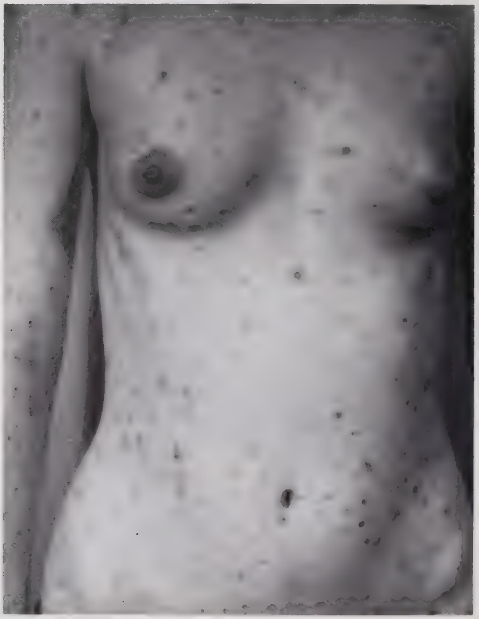

FIG. 16. Generalized nonpruritic symmetrical eruption of secondary syphilis. Maculopapulosquamous syphilids.

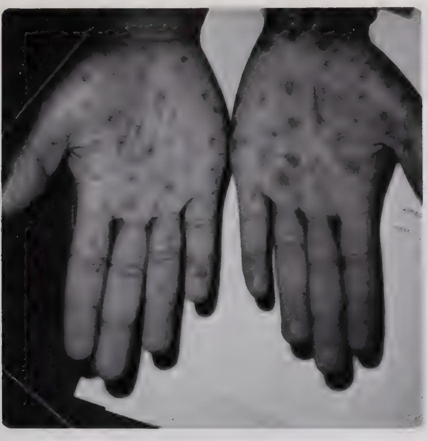

FIG. 17. Secondary syphilis: palmar papulosquamous syphilids.

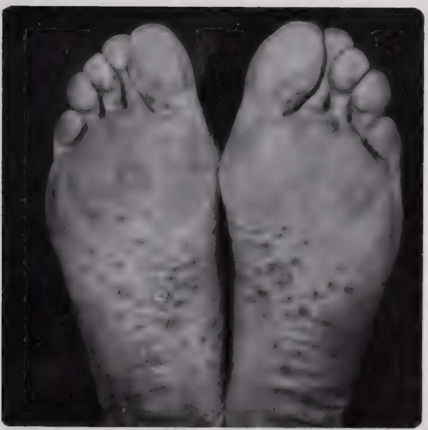

FIG. 18. Secondary syphilis: plantar papulosquamous syphilids.

Mucous membrane lesions may appear in any part of the mouth, pharynx, larynx, the genitalia and in the anal canal. The mucous lesions in the mouth appear as grayish-white patches surrounded by a red areola. Mucous patches and condylomata lata contain large numbers of treponemes and are highly infectious.

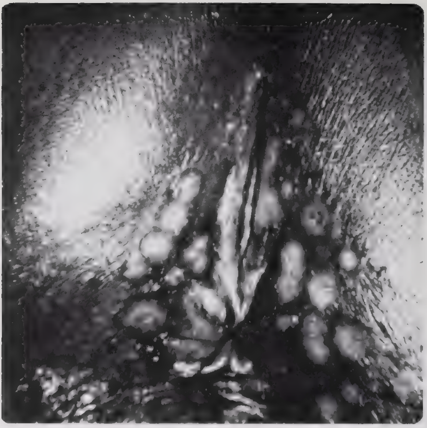

FIG. 19. Secondary syphilis: perianal condylomata lata.

Generalized lymphadenopathy is common in secondary syphilis. The inguinal, epitrochlear, axillary, cervical, occipital, submental and posterior auricular nodes may show some degree of enlargement. Other manifestations of syphilis may include arthritis, eye, liver and spleen involvement. In addition, central nervous system involvement evidenced by meningitis or paralysis of the cranial nerves may be seen in secondary syphilis.

Whenever secondary syphilis is suspected, a darkfield examination of a cutaneous or mucous membrane lesion should be performed and a specimen of blood obtained for serologic testing for syphilis. As a rule, the treponeme may be demonstrated readily particularly from a moist lesion. Failure to demonstrate the treponeme does not, however, rule out a diagnosis of secondary syphilis. The serologic tests, to be discussed, are almost always reactive in secondary syphilis.

As with primary syphilis, signs and symptoms of secondary syphilis will disappear with or without treatment. The untreated or inadequately treated syphilitic patient then enters the latent stage, a stage in which there is no apparent clinical activity of the disease. The patient has no complaints or symptoms relative to syphilis and the physician can find no signs of the disease as a result of his examination. Although ordinarily considered a noncommunicable period, the early portion of this stage (up to 2 years following infection) is potentially infectious because of the appearance of treponeme containing recurring secondary lesions. Syphilis in the late latent stage (beyond 2 years) is not infectious by sexual contact. However, it may be passed on to the fetus by an infected mother during this stage. The diagnosis of latent syphilis is established on the basis of a reactive serologic test for syphilis, after the possibility of other stages of syphilis have been ruled out by physical examination. Cerebrospinal fluid examination, roentgenographic examination of the aorta and ophthalmologic examination to rule out these systemic complications may be utilized in establishing a diagnosis of latency. Latency may continue for the life of the individual (36). In about one third of the untreated cases, the disease will advance to the late stage (37, 38). Late syphilis may have its onset from as soon as 3 to as many as 30 or more years following the primary infection. In most cases, at least 10 years elapse before the pathology of late syphilis becomes manifest. It is divided into three general areas, depending on the system affected—late benign, cardiovascular and neurosyphilis. These divisions of late syphilis are not mutually exclusive for a patient may have more than one type of involvement. A thorough physical examination including roentgenograms and examination of the spinal fluid are essential in diagnosing late syphilis. Fortunately in the United States of America, as elsewhere, late syphilis has now become uncommon.

Congenital syphilis occurs in utero after the fourth month of pregnancy and is divided into stages much like the classification of acquired syphilis. Early congenital syphilis is characterized by the appearance of signs and symptoms up to 2 years of age. Infection for longer than 2 years is considered late congenital syphilis.

The condition of a luetic infant at birth ranges from an apparently normal infant whose infection may not become manifest until much later in life, premature delivery with manifest symptomatology, or death of the fetus with maceration and spontaneous abortion. The signs of early congenital syphilis may include upper respiratory tract infection, rhinitis, generalized lymphadenitis, jaundice, anemia, nutritional deficiencies, celiac disease, and osteochondritis and other bone changes. Nephrosis, hepatosplenomegaly, and neurologic changes may occur as well as pseudoparalysis due to the fact that it is painful for the infant to move as a result of extensive bone involvement. Skin and mucous membrane lesions not only may take on the macular and papular appearance as in acquired syphilis (Fig. 20) but may sometimes be vesicular or bullous. The mucous membranes of the pharynx and nose may produce a mucoid treponeme-laden discharge called snuffles.

The diagnosis of congenital syphilis at birth is not difficult when there are manifest signs of congenital syphilis or when there is a significant titer elevation of a reactive cord serologic test in the newborn compared to the titration level of the mother when reagin tests are employed. A careful physical examination, darkfield examination of accessible lesions and reactive reagin test for syphilis may result in a diagnosis of syphilis. The difficulty in the diagnosis of congenital syphilis in newborns occurs when the only finding is a reactive reagin serologic test which by maternal comparison is not significantly elevated. In this situation, the newborn must be followed with serial monthly quantitative reagin tests for a period of eight months. Noninfected infants will show a gradual fall in titer. A falling titer denotes

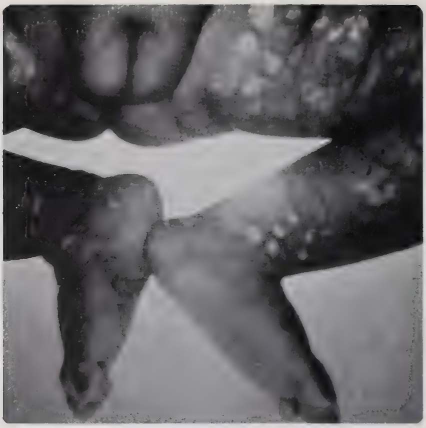

FIG. 20. Congenital syphilis: maculopapular syphilids of the newborn.

passive transfer of the maternal antibody whereas a stable or rising titer indicates active infection.

Late congenital syphilis may exhibit various clinical manifestations (39, 40). These include interstitial keratitis, eighth nerve deafness, dental stigmata of the central incisors (Hutchinson's teeth) (Fig. 21) and the first or 6 years molars (Moon's or mulberry molars), frontal bossing, short maxillas, saddle nose, saber shin, Clutton's joints (a painless synovitis with joint swelling), neurosyphilis. Gummata may occur and lead to perforation of the nasal septum or palate.

Laboratory Procedures: The procedures that may be employed as aids in syphilis diagnosis are listed under table III.

Darkfield Examination: The darkfield examination which was discussed earlier is used for direct identification of Treponema pallidum. The finding of treponemes of typical morphology and motility in the lesions of either primary, secondary, infectious relapse stages or early congenital syphilis constitutes a diagnosis of syphilis. Since many physicians do not have ready access to a darkfield microscope and because this technique is extremely important in diagnosing early syphilis, many municipal health departments will provide this diagnostic service to physicians upon request.

Serodiagnosis: Good medical practice dictates a serologic test for syphilis on every patient. The active and particularly the quiescent stages of syphilis make it necessary to perform serologic tests so as to confirm the diagnosis.

The serologic tests for syphilis are classified as nonspecific or nontreponemal and specific or treponemal (Fig. 22). The nontreponemal tests employ cardiolipin lecithin and cholesterol as the antigen suspension to either flocculate or fix complement in the presence of nonspecific antibodies known as reagins. The specific or treponemal tests utilize live or dead whole treponemes or fractions of treponemes as antigens which will react with treponemal antibodies.

There are two types of nontreponemal tests—the complement-fixation and the flocculation. In the complement fixation test, the antibody (reagin) that

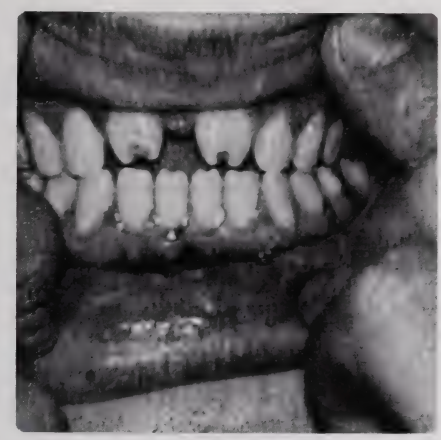

FIG. 21. Congenital syphilis: Hutchinson's incisors showing the "screwdriver" shape with marginal notching and distal narrowing.

TABLE III

Laboratory Procedures for Syphilis Diagnosis

1. Darkfield examination for direct identification of T. pallidum
   a. Lesions of primary, secondary, early congenital or infectious relapse stage.
   b. Aspirate from enlarged regional lymph node.
2. Serologic examination
   a. Reagin serologic tests for screening, assessment of therapy, and congenital syphilis
   b. Confirmatory procedures to detect treponemal antibodies
      Immunofluorescent (FTA-ABS)
      Hemagglutination (MHA-TP)
      Immobilization (TPI). This procedure is no longer available. Specimens will not be accepted for evaluation by the Center for Disease Control in Atlanta, Georgia
3. Cerebrospinal fluid examination for neurosyphilis
   a. Cell count
   b. Total protein values
   c. Cerebrospinal fluid serologic test
4. Biopsy for histopathologic study
5. Roentgen-ray examination for late benign and cardiovascular syphilis

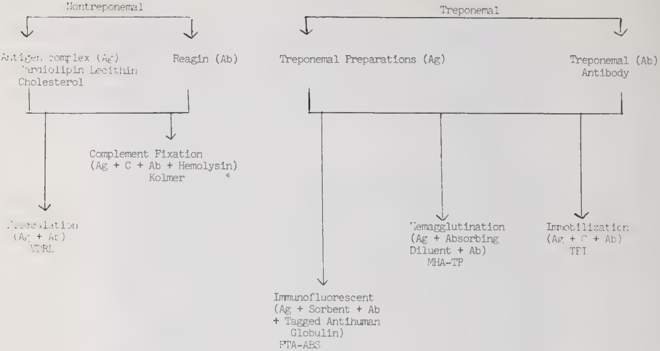
Nontreponemal
Antigen complex (Ag)
Cardiolipin Lecithin
Cholesterol
Reagin (Ab)
Complement Fixation
(Ag + C + Ab + Hemolysin)
Kolmer
Flocculation
(Ag + Ab)
VDRL
Treponemal
Treponemal Preparations (Ag)
Treponemal (Ab)
Antibody
Hemagglutination
(Ag + Absorbing
Diluent + Ab)
MHA-TP
Immobilization
(Ag + C + Ab)
TPI
Immunofluorescent
(Ag + Sorbent + Ab
+ Tagged Antihuman
Globulin)
FTA-ABS

FIG. 22. Serologic tests for syphilis.

reacts with the antigen (cardiolipin lecithin complex) binds the complement and prevents hemolysis of sensitized red blood cells (the indicator system). The most commonly used form of this procedure is the Kolmer test. In the flocculation test, the particles of the antigen complex (cardiolipin lecithin) combine with reagin to form visible aggregates observed as a flocculation. The most widely used test in this category is the VDRL. The Kline, Hinton, and Mazzini tests are similar flocculation procedures. Rapid reagin tests such as the Rapid Plasma Reagin (RPR) and the Unheated Serum Reagin (USR) have been developed for use as screening tests. These screening tests use a modified VDRL antigen suspension made more sensitive by the addition of choline chloride and can be performed on unheated serum or plasma. Rapid reagin tests demonstrating any degree of reactivity should be confirmed by another nontreponemal serologic procedure, such as the VDRL; they are not replacements for the VDRL, Kolmer or other standard procedures when reactive.

The reagin tests lend themselves very well to quantitation. This is accomplished by serial dilutions of the serum or spinal fluid in geometric progression. The titer is expressed as the highest dilution in which the test is fully reactive, e.g., reactive 1:4 means that the serum was reactive at a dilution of 1:4 but only partially reactive or nonreactive at a dilution of 1:8.

Quantitative reagin tests are useful because they establish a base line from which future specimens may be evaluated. Thus, a rising titer may indicate a recent infection, a reinfection, a relapse (i.e., activity of the disease) or an acute false positive reaction. A decline in titer indicates adequate treatment while an unchanged serologic report may indicate serofastness or inactivity of the disease.

As stated earlier, the reagin tests convert to reactive about 4 weeks following infection. Since some chancres take 10 days and most 21 days to develop, a nonreactive reagin test cannot be used to rule out primary syphilis. Reagin titers in primary syphilis may be negative, low or occasionally high (1:32 or higher). By the time the secondary stage is reached, the VDRL is almost always positive, i.e., sensitivity is high in this stage of syphilis. Following treatment of early syphilis, the titer of reactive serum will fall. In primary syphilis this occurs in 4 to 12 months. Treated secondary syphilis will take 9 to 24 months for the titer of the serum to fall. Reagin test results in the latent or late stages of syphilis are variable and the test may remain reactive at low titers permanently. Patients with such titers are considered serofast.

The sensitivity of the reagin tests drops off considerably in late latent and late syphilis. VDRL titers are often nonreactive in neurosyphilis, less often in cardiovascular syphilis. However, in late benign syphilis with active gummata the titers are usually high. Nonreactive results occur in approximately one fourth of the cases of primary, latent and late syphilis (41).

In about 1 per cent of secondary syphilis patients a phenomenon known as the prozone reaction occurs (42). This is a state in which overwhelming amounts of reagin are present in the undiluted test serum but nevertheless flocculation does not take place. As the serum is diluted, flocculation will begin to appear in increasing dilutions. Many laboratories do not routinely perform quantitative reagin tests. Therefore, when syphilis is suspected by clinical evaluation and the reagin test is nonreactive, a second serum specimen should be sent to the laboratory with specific instructions to rule out a prozone reaction.

Although the reagin tests are useful, they may sometimes be reactive in the absence of syphilis, hence "falsely positive" thus decreasing specificity in a number of individuals. This can sometimes reach high proportions depending on the type of test employed and the incidence of syphilis in the population studied. The conditions producing the reaginemia may be of short duration, in which case the serum reverts to nonreactive within 6 months. These are regarded as acute false-positive reactions. (Table IV). Reactivity persisting

TABLE IV

Conditions Reported to Produce Acute False-Positive Reactions (Reactive STS of Less Than Six Months' Duration)

Bronchitis
Chancroid
Coccidioidomycosis
Diphtheria
Hepatitis
Influenza
Jaundice
Leptospirosis
Lymphogranuloma venereum
Malaria
Measles
Mononucleosis
Mumps (angina parotidea)
Pellagra
Pneumonia-bacterial & viral
Pregnancy
Rat-bite fever (sodoku)
Relapsing fever (uncommon)
Rhinitis (common cold)
Scarlatina
Septicemia
Tinea
Trypanosomiasis (uncommon)
Tuberculosis
Typhus
Vaccinia
Varicella (chickenpox)
Vincent's angina (trench mouth)

beyond 6 months in the absence of luetic infection is considered a chronic false positive reaction. Conditions producing these chronic false positive reactions are listed in Table V.

Acute false-positive reactions have been attributed to many viral and bacterial infections, certain vaccinations and immunization, narcotic addiction, aging and pregnancy (43). The greater challenge to diagnosis is presented by the chronic false-positive reactions. They may exist in a number of chronic diseases. They are sometimes ominous in that they may be due to an autoimmune disease such as systemic lupus erythematosus.

The specific or treponemal tests are useful in situations where false-positive reagin tests are suspected. A number of procedures have been developed to test for antibodies that are specific for syphilis. The first test developed was the Treponema Pallidum Immobilization (TPI) test (6). This test measures the ability of the patient's serum plus complement to immobilize T. pallidum that have been cultured in and extracted from artificially infected rabbit testes. This test is expensive, exceedingly difficult to bioassay and perform. It requires rabbit colonies and expert technical help and had been used as a research tool and as a standard for final confirmation. The need for the TPI test is controversial and there are some serologists who feel that it is still the final arbiter in diagnostic problem cases. The Center for Disease Control (CDC) no longer performs this procedure because, for the enormous cost and expertise required, it does not offer any more information than the other confirmatory tests.

TABLE V

Conditions Reported to Produce Chronic False-Positive Reactions (*Reactive STS of More Than Six Months' Duration*)

Addison's disease
Aging (per se)
Atopic dermatitis
Autoimmune disorder
Bejel (biologic true reaction)
Brucellosis
Cirrhosis of liver
Collagen disorder
Connective tissue disorder
Cryoglobulinemia
Dermatomyositis
Diabetes mellitus
Dysproteinemia
Epilepsy
Erythema nodosum
Glomerulonephritis
Hashimoto's thyroiditis
Hemolytic anemia
Histoplasmosis
Idiopathic thrombocytopenic purpura
Leprosy
Lupus erythematosus
Lymphatic leukemia
Lymphosarcoma
Major allergic conditions
Malaria (usually acute BFP)
Metastatic carcinoma
Multiple myeloma
Myocardial infarction
Narcotic addiction
Pemphigus vulgaris
Periarteritis nodosa
Pernicious anemia
Pinta (biologic true reaction)
Rheumatic fever
Rheumatoid arthritis
Sarcoidosis
Scleroderma
Subacute bacterial endocarditis
Tuberculosis (also acute BFP)
Yaws (biologic true reaction)

The most commonly used specific or treponemal test is the FTA-ABS test (41). This is an indirect fluorescent test in which suspected serum is brought into contact with lyophilized T. pallidum that has been fixed to a slide. A fluorescein-tagged conjugate of anti-human globulin is overlayed on this mixture. If the serum contains specific antisyphilitic antibodies, the treponemes will be coated with the fluorescein and can be detected by fluorescence under an ultraviolet microscope. The specificity of the test is increased by diluting the patient's serum 1:5 and absorbing it with an extract from the Reiter strain. This removes any group antibody directed against indigenous treponemes, such as T. microdentium.

In addition to the TPI and the FTA-ABS tests, another confirmatory procedure that is gaining popularity because of lower cost and ease of performance is the hemagglutination assay for treponemal antibodies. Rathlev (44) described the first hemagglutination test which employed sheep red blood cells sensitized with ultrasonicated material from pathogenic T. pallidum (Nichol's strain). Antitreponemal antibody was indicated by hemagglutination. Later modifications of this procedure introduced an absorbing reagent so as to remove cross-reacting antibodies. Microhemagglutination methods for which reagents are now available commercially have also been developed (45, 46, 47). Microhemagglutination tests are designated as MHA-TP and the procedure is based on the earlier macrovolume, passive hemagglutination tests (designated as TP-HA).

The three confirmatory procedures (immobilization, immunofluorescent and microhemagglutination), commonly yield reactive results with nonvenereal treponematoses such as yaws, pinta and bejel. Therefore, it is impossible to diagnose latent syphilis with the available serologic tests in patients who have been exposed to other treponematoses and present no clinical evidence of syphilis.

A comparison of the reactivity of these three procedures (Table VI) suggests that the TPI test is slightly less sensitive than the FTA-ABS (41) and the hemagglutination tests in all stages of syphilis (48) but has high specificity. However, the presence of antibiotics in the serum specimens and antioxidants in the stopper of specimen tube are treponemicidal and will inactivate motile

TABLE VI

Range of Reactivity of VDRL, TPI, MHA-TP and FTA-ABS Tests during Various Stages of Syphilis (Treated and Untreated)

% Reactive

	VDRL	TPI	MHA-TP	FTA-ABS
Primary syphilis	78.	56.–67.	64.–94.	82.–100.
Secondary syphilis	97.	94.–100.	96.–100.	99.–100.
Latent syphilis	74.	94.–97.	95.-100.	95.–98
Late syphilis	77.	92.–98.	95.-100.	95.–100

Data adapted from tables presented by Deacon et al (41), Logan and Cox (46), and Coffey et al. (47).

treponemes, thus yielding a false-positive report. The FTA-ABS test becomes reactive earlier in the disease than the other serologic tests. The MHA-TP appears to be equal in sensitivity to the FTA-ABS test in all stages of syphilis except primary syphilis (46, 47) (Table VI). The sensitivity of the microhemagglutination test may be greater than that of the FTA-ABS test in treated cases of latent and late syphilis (46).

There is evidence that both the FTA-ABS and the hemagglutination tests may be falsely positive infrequently in certain patient groups. The conditions reported as possibly associated with false-positive FTA-ABS tests include rheumatoid arthritis, lymphosarcoma with autoimmune hemolytic anemia, alcoholic cirrhosis, systemic lupus erythematosus, pregnancy, smallpox vaccination and antinuclear antibodies (49) (Table VII).

Treponemal hemagglutination tests infrequently may be false positive in patients with connective tissue disease, leprosy, pregnancy (50), and infectious mononucleosis (51). Because the hemagglutination tests are a fairly recent addition for the serodiagnosis of syphilis and have not undergone extensive clinical use it would not be surprising to find that further application of this procedure will probably reveal other conditions that may give false positive results for syphilis.

The immunofluorescent and hemagglutination tests have a high degree of specificity even though they may probably produce false-positive reactions in a small percentage of cases. Both tests remain reactive for many years whether the syphilitic infection is treated or not. Thus, these tests do not provide information regarding the clinical activity of syphilis or necessity for treatment. The best test for following response to treatment is a quantitative reagin test such as the VDRL. Since the reagin test may be nonreactive in as many as a third of the cases of late syphilis, use of the more sensitive FTA-ABS or hemagglutination test becomes necessary when central nervous system or cardiovascular syphilis is clinically suspected in any patient. In situations where the only presumptive evidence of syphilis is a reactive reagin test, either the FTA-ABS or hemagglutination test may be employed. If the primary stage of syphilis is suspected, it is advisable to perform the FTA-ABS test which is more sensitive than the hemagglutination test in this stage of syphilis. If

TABLE VII

Conditions Reported as Possibly Associated with False-Positive Confirmatory Tests for Syphilis

FTA-ABS	Hemagglutination
Rheumatoid arthritis	Connective tissue disease
Rheumatoid factor	Leprosy
Lymphosarcoma with autoimmune hemolytic anemia	Pregnancy
Alcoholic cirrhosis	Infectious mononucleosis
Systemic lupus erythematosus	
Drug-induced lupus erythematosus	
Pregnancy	
Vaccination	
Antinuclear antibodies	

serologic results are still equivocal after applying and repeating the confirmatory tests, laboratory tests for conditions that are associated with false-positive confirmatory tests may be ordered. In the final decision, the physician will have to rely upon clinical judgment for diagnosis.

A modification of the FTA-ABS test, the FTA-ABS (IgM) has been developed for use in the diagnosis of congenital syphilis in the newborn (52). The FTA-ABS test, which detects IgG antibodies, is modified by substituting antihuman IgM conjugate for the IgG conjugate. Under normal conditions only IgG is transferred across the placenta. The fetus and neonate can produce IgM antibodies in response to (syphilitic) infection. This test is based on the assumption that since IgM antibodies do not traverse the placenta, any IgM antibody detected by the FTA-ABS (IgM) test from neonatal sera has to be attributable to infection in the newborn.

However, questions relative to the sensitivity and specificity of the FTA-ABS (IgM) test have been raised by scientists at CDC (53). A critical review by Kaufman and his colleagues (54) of available published data indicates that this test is insufficiently sensitive for use as a screening test in infants with delayed onset of symptoms. Additionally, nonspecific interference in the mechanism of the test may be introduced by the production of IgM anti-IgG antibody by the infant. The IgM anti-IgG attaches to IgG specific to T. pallidum which may be present by placental transfer and is detected by the IgM specific immunofluorescent conjugate. The IgM detected in the test may not be specific for the infecting agent. As a result of these findings and until the sensitivity and specificity of the FTA-ABS (IgM) test is improved, CDC has discontinued routine performance of this test and is recommending serial quantitative VDRL tests for evaluation of newborns potentially at risk who fail to show characteristic signs at birth.

Cerebrospinal Fluid Examination: There are three tests of the cerebrospinal fluid (CSF) that are essential for the diagnosis of neurosyphilis:

1. Cell count. More than 4 lymphocytes per mm^3 is regarded as abnormal.
2. Total protein. Protein is always elevated in active neurosyphilis. Normal values may vary in different laboratories. Generally though, total protein values over 40 mg% are abnormal.
3. Seroreaction for syphilis. Cerebrospinal fluid may be tested either by reagin or FTA procedures. However, the specificity of the FTA procedure for cerebrospinal fluid (FTA-CFS) test has not been established. The Center for Disease Control is currently undertaking a large cooperative study to evaluate the specificity and sensitivity of this procedure. Pending the outcome of this study, CDC has recommended that the test not be performed since it cannot be adequately interpreted.

A reactive cerebrospinal fluid reagin test is practically always an indication of neurosyphilis but not necessarily of its activity. Activity of the disease is gauged by the cell count and protein values both of which may rise with any condition which causes meningeal irritation. However, in the presence of a reactive cerebrospinal fluid reagin test, lymphocytes and total protein above normal values are indicative of active neurosyphilis.

Although highly specific, the cerebrospinal fluid reagin test may be falsely nonreactive in many cases of neurosyphilis. Hooshmand et al (55) found reactive cerebrospinal fluid reagin tests in 56.7% of his neurosyphilitic patients (total studied 176) whereas the cerebrospinal fluid FTA-ABS test was reactive in 156 patients.

Histopathologic Diagnosis

Histopathologic study of the syphilitic lesion may aid in confirming a diagnosis of early syphilis when the darkfield examination is negative or in differentiating a late syphilitic lesion from other conditions such as tuberculosis. The syphilitic lesion presents distinctive histologic features. In primary syphilitic lesions, lymphocytes and plasma cells infiltrate the corium chiefly around lymphatics and blood vessels. Endarteritis in the small blood vessels causes hypertrophy of the endothelium which obliterates the lumen. Due to the reduced blood supply the surface of the lesion erodes. It is possible to demonstrate Treponema pallidum within these lesions by staining with Levaditi's silver stain. The organisms may be found in the capillary and lymph vessel walls.

In secondary lesions other than the macular syphilid which seldom presents a characteristic picture, there is endothelial swelling of the superficial and deeper vessels in the corium where perivascular infiltrates with numerous plasma cells are found. T. pallidum may be seen in great numbers in condylomata lata lesions.

In the tertiary stage, one sees granulation tissue consisting of lymphocytes, plasma cells, histiocytes, fibroblasts and epithelioid cells. Pronounced vascular changes particularly in the larger vessels are seen which are often obliterated by endothelial proliferation. In short, tertiary syphilitic lesions are characterized by a preponderance of plasma cells and giant cells of foreign body type and characteristic vascular changes.

Infectivity test: Mention should be made of the infectivity test in which syphilitic infection can be established in mature male rabbits by intratesticular inoculation of clinical specimens. It is mainly a research procedure for it entails maintenance of an animal colony. Its use as a diagnostic test is limited because of the long incubation period, the scarcity of treponemes in the late stages of syphilis, and the natural contamination of rabbits by T. cuniculi.

Brief Recapitulation On Laboratory Diagnosis: The diagnosis of gonorrhea is presently by cultural examination with confirmation by fermentation reactions. A direct smear examination will suffice in the male patient with acute uncomplicated urethritis. However, a culture is warranted if the smear examination is negative and gonorrhea is suspected. The application of serologic methods for gonorrhea diagnosis is still under investigation.

For syphilis, the darkfield examination is, of course, par excellence for infectious syphilitic lesions. This examination if done by a competent examiner will offer a definitive diagnosis if positive. However, negative darkfield examinations do not rule out syphilis. The histologic characteristics of syphilitic lesions are quite distinctive and may aid the diagnosis particularly if treponemal organisms are demonstrated within the specific lesions. Spinal fluid examinations in neurosyphilis and x-ray and fluoroscopy in cardiovascular involvement are aids in confirming the diagnosis.

The serodiagnosis of syphilis is extremely important particularly for latent syphilis where the patient does not present any signs or symptoms of syphilis. The reagin tests are not absolutely specific for syphilis but are effective in screening patients. Conditions under which these tests may be falsely positive have been listed. Nevertheless, they are sensitive, simple to perform, practical, inexpensive and widely available. The specific or confirmatory tests such as the FTA-ABS and hemagglutination, are useful for resolving false-positive cases and for detecting a larger number of early and late syphilis patients than do the reagin tests. However, the hemagglutination tests are not as sensitive as the FTA-ABS test in primary syphilis. Presently, the only confirmatory test providing a high degree of sensitivity and specificity in all stages of syphilis is the FTA-ABS.

The serologic diagnosis of syphilis, although improving continuously, is still imperfect and in a small number of cases these tests are still liable to error, so that the ultimate diagnosis in problematic cases must depend upon assessment of all the facts of the case and the judgment of the physician.

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Human Gastric and Intestinal Response to Meals Studied by a Multiple Indicator Dilution Method

HENRIK O. LAGERLÖF

Guest Editor

with

Catja Johansson and Kerstin Ekelund

Human Gastric and Intestinal Response to Meals Studied by a Multiple Indicator Dilution Method

HENRIK O. LAGERLÖF*
GUEST EDITOR

WITH THE COLLABORATION OF

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HENRIK O. LAGERLÖF, CATJA JOHANSSON, AND KERSTIN EKELUND

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Preface

The transit of the intestinal contents has three dimensions in space and at least two in time, mean transit time and clock time. Their mutual actions are, therefore, not easy to describe both briefly and intelligibly. The first manuscripts from the late sixties were accordingly limited.

We owe many thanks to the Editorial Board of the *Scandinavian Journal of Gastroenterology* for the opportunity they gave us for publishing the first rather theoretical paper about the method. We also thank the Editor, Docent J. Myren, for his permission to publish figures which originally appeared in his Journal.

This book would not have been written if K. Wormsley had not found our work interesting and invited me to speak on the subject at the meeting of the European Pancreatic Club in Dundee, Scotland in 1974. It was at that meeting that David Dreiling, appreciating the time and space necessary to penetrate the problems involved to get a deeper understanding, exhorted me to write this monograph for *The Mount Sinai Journal of Medicine*. My collaborators and I are greatly thankful for this opportunity.

In a work of this scope it was, of course, impossible to cite all the literature of even a major part of it. References have been selected for this historical significance, their usefulness, and, too often perhaps, their availability.

The work my collaborators and I describe in this book would not have been possible without the help and assistance of many persons. We want to thank: Professor L. E. Böttiger, present Head of the Department of Medicine, Karolinska Sjukhuset, for his interest and willingness to put resources at our disposal; the Laboratory of Gastroenterology, Karolinska Sjukhuset; Nicholaas Kulsdom, Ljubinca Kostić, Ingemar Larsson, Liss Nordberg, Kristina Strömbeck, Kristina Virtanen, and Ragnhild Östman for their professional competence and loyalty; Alf Bergin, Ulf Brodin, and Elisabeth Berg for compilation and translation of computer programs and help with statistical evaluation of data; M-d. Ahlberg; and Britt Andersson for excellent work in typing the manuscripts.

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HENRIK LAGERLÖF
Guest Editor

Introduction

The integrated function of the human gastrointestinal channel after food intake has not been studied using quantitative methods. Even the partitive functions are incompletely known. The present concept of postprandial intestinal flow volumes and absorption is based on semiquantitative studies. Data on the gastric secretion in response to food rely on indirect methods; and data on the gastric emptying of different food components are scarce. To formulate a hypothesis as to how stomach, intestine and related organs interact to regulate the body caloric supply, quantitative information must be made available concerning the time course of gastric emptying and intestinal absorption after intake of meals of ordinary composition. Such information presupposes a method by which the postprandial flow of intestinal chyme can be determined during several hours after feeding. Techniques used for flow measurements in circulatory work have been applied to the intestine but give only limited information, since in the intestine the fluid flow is not constant, but dependent on several time-dependent variables—namely, the gastric emptying rate, the intestinal secretion and absorption, and the transit time and volume in the investigated segment.

A multiple indicator dilution method for quantitative studies in the upper gastrointestinal tract has been developed (1–3). The method permits determination of the intestinal flow rate during several hours after food intake in man, irrespective of variations in intestinal volumes and gastric emptying. The transport times of the intestinal chyme through the upper intestine can be assessed and consequently the gastric variables. The validity of the method was found to be high, when tested in an in vitro model of the stomach and intestine (4). Previously reported results from two series of experiments (5–10) demonstrated the necessity of investigating meals of different composition.

This presentation aims at a description of the multiple indicator dilution technique, the mathematical background of the method, and an analysis of how the gastric and intestinal response to food is influenced by the composition of a meal.

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I. Related Contemporary Methods for Studies of Human Gastrointestinal Functions—Theoretical and Methodologic Remarks

CATJA JOHANSSON

Indicator dilution techniques in the intestine

The method of determining the mean transit time from the dilution of an indicator, according to the principles introduced for circulatory work by Hamilton, Moore, Kinsman, and Spurling (1), was first applied in the human intestine by Dillard, Eastman, and Fordtran in 1965 (2) and later was used by Whalen et al (3), Barreiro et al (4), and by Bernier and his collaborators (5). The usual practice is to introduce a triple lumen tube into the intestine. Through the proximal openings, a test solution containing a nonabsorbable indicator is infused at a constant rate. Through a second tube ending 10–15 cm distal to the first, single or repeated instantaneous injections of a second nonabsorbable indicator are given. Through a third tube ending at a varying distance to the proximal, timed collections of fluid are made. A dilution curve is constructed by plotting the time on the abscissa against the concentrations of the instantaneously injected indicator in samples at exit of the segment on the ordinate. The distance in time from the moment of injection to the center of gravity of the dilution curve is the mean transit time for the indicator to transit the investigated segment, if flow and volume are constant. The mean transit time (Tm, min) can then be calculated according to the formula given by Hamilton et al:

$$Tm = \frac{c_1t_1 + c_2t_2 + \cdots + c_nt_n}{c_1 + c_2 + \cdots + c_n} \qquad \textit{Equation 1}$$

where $c_1, c_2 \cdots c_n$ are concentrations of the indicator in collected samples at $t_1, t_2 \cdots t_n$ minutes after the injection and n is the last sample in which the indicator is present. Since, as first recognized by Hamilton and Remington (6), the temporal events are identical between the concentration curves obtained after an instantaneous injection or a continuous infusion of an indicator, the Tm may equally well be derived from the accumulated concentrations of the indicator given at constant rate.

Likewise, the rate of fluid flow at the level of sampling (f_s, ml/min) may be derived from the amount of injected indicator divided by the area of its dilution curve, or from the dilution of the continuous infused indicator after an established equilibrium according to:

$$f_s = \frac{f_i \cdot c_i}{c_s} \qquad \textit{Equation 2}$$

where f_i is the infusion rate (ml/min), c_i the concentration of indicator in the test solution and c_s the concentration of indicator at the sampling site.

Provided that the test solution is not changed by absorption or secretion during transit, and that irregular contamination of endogenous material is avoided, the volume of the segment (V, ml) may be derived from

$$V = f_s \cdot T_m \qquad \textit{Equation 3}$$

For descriptive illustrations of the formulas the work by Zierler (7) should be consulted.

It is essential for the validity of the equations when applied to intestinal studies that conditions of steady state be established. This implies that an indicator infused at a constant rate shall pass at the same rate at the sampling site. Because of incomplete sampling, usually by siphonage, this criterion is not suitable in the experimental work. A steady state is therefore assumed to prevail when the concentrations of the continuously infused marker do not differ by more than a few percents in repetitive samples from the exit of segment.

A further sign of an established steady state is a strict semilogarithmic decline of the descending part of the concentration curve obtained for the instantaneously injected indicator in samples from the end of segment. A rejection of deviating curves has usually been made in the calculations.

By including mannitol in the test solution, Dillard et al minimized the changes of the flow rate along the segment. They were able to demonstrate that at flow rates between 3 and 7 ml/min the transit time varies little and the intestinal volume increases proportionally to an increasing flow rate. At higher inflow rates the intestine responds with a decreasing transit time and the volume remains constant.

A comparison of results reached by different workers (8) showed only slight variations in the mean transit times 11.8 ± 5.1 min to 16.0 ± 7.1 min through 25–30 segments of jejunum, when salt solutions of similar composition were infused at inflow rates of 8 to 10 ml/min.

The segmental perfusion techniques have been developed in recent years. The problem of contaminating endogenous contents has been approached by a double segmental perfusion technique (9) or by attempts to close off the test segment with balloons. The technical problems, the relations between lengths of segments, lengths of equilibration periods and composition of test solutions have been analyzed and comprehensively reviewed by Modigliani, Rambaud, and Bernier (10).

A recent development of the indicator dilution techniques was presented in 1973 by Meeroff, Go, and Phillips (11). The method implies the duodenal recovery of one intragastric and one intraduodenally infused indicator and permits quantitative determination of secretion, absorption and transit under conditions of constant volume and flow. A remarkable application of the method was made by the Mayo Clinic Group (12) studies of the daily excretion of bile salts and bilirubin.

Intestinal absorption

Much attention has been paid to the advantages and difficulties of segmental perfusion techniques in studies of the absorptive processes (5, 13). In recent years a rapidly increasing number of studies have been focused on the absorption kinetics of water (14), amino acids (15), mono- and disaccharides (16) in different segments of the intestine.

It is probable, that the use of an experimental set-up with high perfusion rates, short intestinal segments and poorly absorbed substances facilitates the establishment of a steady state. These same experimental conditions do, however, limit the reliability of the method for studying the intestinal response to nutrients and make a translation of the results in terms of absorption after food intake difficult.

One variable involved in the absorptive process seems to have been little recognized in these studies although suggested already in 1968 by Barreiro, McKenna, and Beck (17). This is the time during which absorption takes place. The absorption time should be equal to the time of contact between the nutrient examined and the intestinal mucosa, i.e., to the time for an infused portion to transit the segment. In segmental perfusion studies this time is assumed to be identical with the time periods during which the samples from the exit of segment are siphoned. No objection can be raised if the results are expressed in terms of absorbed amounts against loads, since both increase linearly with lengths of the sampling periods if steady state conditions prevail, and the length of segment is fixed and one test solution is used.

If, however, the results are expressed in terms of absorption rates or fractional absorption rates, an error is introduced which can be considerable if the investigated segments are long and the sampling period short. It is likely that this error should increase by the use of low perfusion rates and of easily absorbed substances. If the time during which the absorption takes place is not known, comparisons between the absorption rates of different test substances or of the same test substance at different loads have to be evaluated with care. Different transit times through the segment are apt to occur and may result in similarities or dissimilarities that are not related to the absorbability per se.

Since the work published in 1957 by Borgström, Dahlqvist, Lundh, and Sjövall (18), little has been added to the knowledge concerning the absorption of different food constituents after feeding in man. By the use of an intubation technique they demonstrated that the absorption of a composite meal was almost completed in the upper part of the jejunum. The percentage absorption was determined from comparison of the ratios between nutrient to marker in the test meal and in samples of intestinal juice collected by siphonage at different intestinal levels. This method of calculating the absorption of fat was criticized by Wiggins and Dawson (19) because fat separates from a water-soluble marker in the stomach. By substituting the test meal ratios for those in samples from the proximal duodenum, Borgström et al (20) were later able to establish their results. With this semiquantitative technique, the average absorption efficiency of the components in the meal may be assessed and related to the length of the intestine, whereas in studies of the dynamics and the time course of the absorption of a meal, the gastric emptying of the nutrients and the intestinal transit must be quantitatively followed.

The results on the site of protein absorption have later been modified in studies of Nixon and Mawer using the same technique (21, 22); they estimated the endogenous contribution of protein by comparing the amino acid pattern in the chyme to that in the meal, and found that the absorption of dietary protein was completed at a higher intestinal level than that recorded in the study by Borgström et al.

Gastric emptying and secretion

Of the methods studying the process of gastric emptying, the serial test meal introduced by Hunt (23) has contributed most to our present knowledge. This method implies quantitative recovery of the gastric contents at time intervals after ingestion and repeated experiments are needed to construct an elimination curve of a test meal from the stomach. By addition of a nonabsorbable indicator the gastric volume and secretion can be assessed. The serial test meal presupposes a high degree of individual reproducibility to allow a quantitative analysis of the elimination curves. In recent years Hunt and collaborators have directed their work toward the determination of the factors influencing the early phase of gastric emptying (24, 25, 26, 27).

To circumvent the need for repetitive experiments the double sampling technique was proposed by Salamanca et al (28) and developed by George (29). A dilution indicator is injected into and sampled from the gastric contents at time intervals during the emptying of a liquid meal, which may contain a second marker. Homogenicity of the gastric contents is claimed by continuous withdrawal and reinstallation of the contents, whereby the influence of the artifical mixing on the emptying is assumed to be moderate. A further development of the method was recently published by Moberg, Carlberger, and Báràny (30), who reported the advantages of the method in studying the emptying of composite meals.

Although intubation procedures, since the work of Shay and Gershon-Cohen (31), are thought not to affect the process of gastric emptying, they are time-consuming and may be unpleasant for the patient. The problem is avoided in the scanning techniques (32, 33) by which the elimination of a radioisotope from the stomach is determined by external counting. The elimination curve can be constructed with great accuracy, provided the changing gastric volumes and amounts of isotope in neighboring organs are controlled. Against the claimed advantage that the isotope may be incorporated into solid food remains the fact that the obtained elimination curves represent the emptying of the isotope and give limited information as to the fate of the ingested food.

Another approach to quantitative studies of gastric emptying is based on the examination of the emptied contents. In draining the stomach through a duodenal fistula, Thomas, Crider, and Morgan observed a rapid gastric emptying which could be retarded by reinstallation of the gastric effluent into the duodenum (34). Drainage of the stomach is therefore suitable in studies where the inhibitory influence from the proximal intestine is to be avoided, but is unsuitable in studies of the gastric emptying in response to food. The same limitation applies to the double indicator dilution technique recently introduced by Meeroff, Go, and Phillips (11) in which the duodenal contents are continuously siphoned at the ligament of Treitz. The possible consequences of withdrawing the intestinal contents are further discussed below.

In studies of normal gastric function, interest has been focused on:

1. The relationship between gastric volumes and the rate of emptying; for an inert solution gastric distention is regarded as the only way to enhance the emptying rate.

2. The relationship between the composition of a meal and the rate of its emptying from the stomach, i.e., the mechanisms by which the gastric emptying rate is regulated.

The extensive work by Hunt and collaborators has resulted in hypotheses concerning the existence of duodenal receptors which are sensitive to acid, osmotic pressure, and products of fat digestion and which operate to control the rate by which gastric contents are delivered to the intestine. The degree to which these receptors are activated is supposed to be related to the concentrations of the substances in the gastric effluent and in duodenal and intestinal contents (35).

It is to be expected that the degree of receptor activation is related to the mucosal area exposed to the substance. This area is determined by the gastric delivery rate, the velocity with which the intestinal contents are propelled, and the elimination rate of the substance from the intestinal lumen (or, in case of acids, to the neutralization rate). The extent to which the receptors operate after ingestion of composite meals is incompletely known, since until recently quantitative studies have been confined to test meals made up of single components. In 1974 Moberg and Carlberger (36) reported results from different composite meals and concluded that the fat content of their meal was the most important determinant of its emptying rate. Little is known about how the inhibitory influence is mediated to the stomach, and how far down the intestine it may be evoked after food ingestion.

Much effort has been made to describe the characteristics of the gastric elimination of the test meal marker. The elimination curve gives information on the emptying of the meal, provided the ratio between marker and all components of the meal remains constant during the emptying process. No reliable conclusions can, however, be drawn from the curve concerning the emptying of the gastric contents (meal plus secretion), which is the more correct resultant of the gastric propulsive activity.

The determination of the gastric secretion of hydrochloric acid in response to food has not been made using direct methods. By estimating the body base excess after a steak meal, Rune (37) concluded that the first hourly gastric secretory response was of comparable magnitude to that obtained after stimulation with gastrin analogue. Another approach by Wyllie, Boulos, Lewin, Stagg, and Clark yielded the interesting information that the gastric secretion proceeded at a constant rate for one hour after the withdrawal of an ingested meal (38).

Since 1942, when Lagerlöf introduced a method for estimation of the biliary and pancreatic secretion, this technique and its modifications have been widely used to describe the pancreatic response both to hormonal stimulation and to various meals (39).

Gastrointestinal hormones

The rapidly growing field of gastrointestinal endocrinology must be mentioned, although the determination of the serum levels of enterohormones was not yet included in the method at the time of the experiments here presented.

The finding of Mutt and Jorpes (40) that the cholecystokinetic and pancreas-enzyme-stimulating properties of their cholecystokinin preparation followed each other through every step of purification was the starting point for a development in the knowledge of gastrointestinal functions. Grossman (41) was the first to grasp its significance and soon found that all intestinal hormones had an influence on secretory and motor functions in addition to their classic effect. The discovery of mutual relation between the enterohormones further complicated the picture.

The identity of the enterohormones that take part in the gastrointestinal action on ordinary food, and how they are related to and interrelated with inhibitory or facilitatory neural mechanisms are not known. In studying the serum levels of the enterohormones after food intake in man the gastric emptying and intestinal absorption have not been concomitantly controlled.

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II. Mathematical Analysis of Indicator Concentration-Time Curves Obtained in Systems with Constant or Varying Mean Transit Times

HENRIK O. LAGERLÖF

The goal of the indicator dilution methods hitherto used has been to determine the rate of flow in systems with constant volume and flow. By determining the mean transit time it also has been possible to assess the volume (Chapter I, Equation 3).

In our experiments both goals and conditions have been different. We have been interested not only in the flow of water but also in the flow and timing of indicator particles as they mark the contents leaving the stomach. The conditions in the intestine are, furthermore, much more complicated than in a stationary system. We deal with time-related variations of gastric input as well as passive and active changes of intestinal capacity, secretion and absorption. To this is added a rather irregular motor function.

Methods

Human experiments. A detailed description is given in Chapter III. In short, the technique requires intubation of the upper gastrointestinal tract with three tubes, which are swallowed the day before an experiment. When in correct position the tubes end in the gastric antrum, in the descending duodenum, and in the jejunum, 70 cm distal to the pyloric sphincter (Fig. 1).

A liquid test meal, to which polyethylene glycol (PEG) has been added is given to drink. Samples from the gastric antrum are withdrawn at intervals.

Starting at the time of ingestion, the duodenal contents are indicated by a continuous infusion of three marker substances, infused into the duodenum at a constant rate (0.5 ml/min) one at a time, with change of indicator every 40th minute. The jejunal contents are continuously siphoned in 20-minute samples.

In vitro experiments. To be able to change the variables independently of each other, we used an intestinal model. A 750-mm-long collodium tube with a circumference of 56 mm acted as the intestine. It was attached in a sine-wave configuration to a wooden plate (Fig 2), which was fastened on a rocking device making 15 oscillations per minute around the long axis of the tube. The rocking motion was intended to simulate the segmental movements of the intestine, and together with the sine-wave attachment of the tube it caused mixing of tube contents. A piston pump, which could be adjusted to give different inflow rates, was attached to the proximal part of the tube. The pump drained a container which functioned as "stomach." The "stomach" contained a stirring device and a solution of polyethylene glycol (PEG) in water. This solution was continuously diluted to simulate gastric secretion.

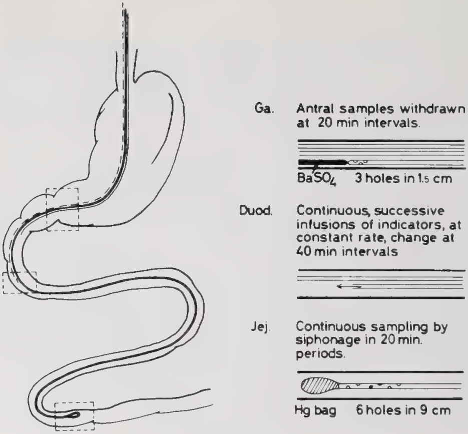
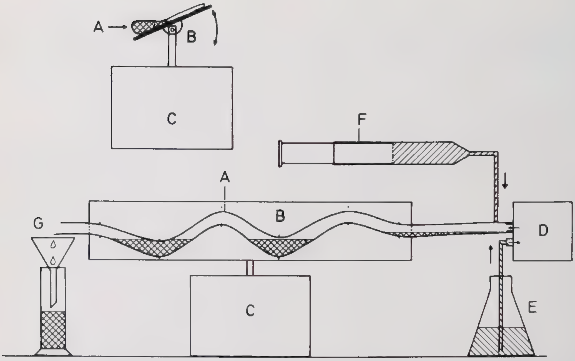

Ga.
Antral samples withdrawn at 20 min intervals.
BaSO4
3 holes in 1.5 cm
Duod.
Continuous, successive infusions of indicators, at constant rate, change at 40 min intervals
Jej.
Continuous sampling by siphonage in 20 min. periods.
Hg bag
6 holes in 9 cm

FIG. 1. Position of the tubes.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E

FIG. 2. Intestinal model. A: Collodium tube; B: Rocking wooden plate; C: Rocking wooden device; D: Piston pump; E: Container with polyethylene glycol, PEG, solution; F: Syringe with indicator; G: Sampling site.

The indicators were infused through a thin plastic tube just distal to the pump, in the same way as described for the human experiments.

By adjusting the piston pump to different inflow rates and by elevating or lowering the distal part of the tube, changes of tube volumes and flows at the sampling point could be induced. Flow volumes from the tube were collected in 10-minute periods.

Definitions and symbols

Gastric and jejunal concentrations of all markers were transferred into a common relative unit/ml. Thereby, the amount of duodenal indicator infused during one jejunal sampling period was given the magnitude of 100 units. Denote that a sampling period is 20 minutes in human experiments and 10 minutes in in vitro experiments, and hence 200 units and 400 units respectively are infused during a duodenal indicator run.

c_n	individual indicator concentration in jejunal sample *n* (units/ml)
c_n^P	concentration of PEG in jejunal sample *n* (units/ml)
C_n	sum of individual indicator concentrations in jejunal sample *n*, i.e., concentration of total indicator (units/ml)
f_n	jejunal flow in period *n* (ml)
T_m	mean transit time of an indicator, i.e., the mean time it has taken the indicator particles to pass from the site of infusion to the site of collection (min)
tm_t	mean transit time of the particles of total indicator, which passes the sampling site at the time *t* (min)
tm_n	average time it has taken for the amount of total indicator that passes the sampling point during a sampling period to transit from the site of infusion to the site of collection (min)
tm_t^N	mean transit time of the particles of indicator *N*, which passes the sampling site at the time *t* (min)
tm_n^N	mean transit time for the amount of indicator *N* that passed the sampling point during period *n* (min)
$\Delta f_n C_n$	change of flow of total indicator during period *n*
Δtm_n	change of *tm* for total indicator during period *n* (min)
Δtm_n^N	change of *tm* for indicator *N* during period *n* (min)
I_m	midinfusion time, i.e., 20 minutes after start of the infusion of an indicator (min)
a^N	appearance time for an indicator, i.e., the time from the start of infusion of the indicator until the first indicator particle appears at the sampling site (min)
a_n	appearance time for total indicator in period *n* extrapolated from a according to text (min)
Pm	mean passage time, i.e., the average time it takes the particles of an indicator to pass the sampling point (min)
pm_n	mean passage time for total indicator in period *n* (min)

Analysis of indicator concentration time curves under different experimental conditions

Constant volume and flow. Figure 3 shows the indicator flow-time curve in a model experiment in which fluid was infused continuously in the artificial intestine, whose volume was kept constant. After equilibrium of in- and outflow of water, indicators were infused one at a time as described above. Indicator began to appear after an appearance time of some minutes. After the

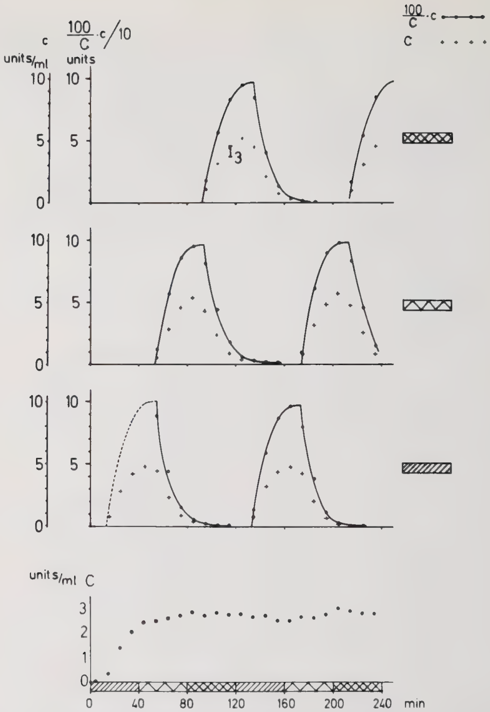

FIG. 3. Model experiment with constant volume and flow. The shadowed areas at the bottom refer to the constant infusion of different indicators at the "duodenal" level, the points to concentrations at the "jejunal" level. 10-minute periods.

appearance time plus the infusion time, i.e., after about 40 minutes, the concentration of total indicator increased very little, i.e., equilibrium between in- and outflow of indicator was obtained. The outflow of water per period may then be determined from $100/C_n$.

Further the sum of 100 c/C in consecutive periods, during which an indicator pass at the sampling site is equal to 400 units, i.e., to the amount infused during one duodenal indicator run.

Plotting the indicator flow against time gives identical curves for the different indicators (Fig. 3). The descending part of the curve is a decaying exponential (Fig. 4) and when 10-minute periods are used we get:

$$\frac{\dfrac{100}{C_{n+1}} \cdot c_{n+1}}{\dfrac{100}{C_n} \cdot c_n} = 10^{-k \cdot 10} = K \qquad \textit{Equation 1}$$

where the indices n and $n + 1$ are the numbers of two adjacent periods and k and K are constants.

For the ascending part of the curve we get similarly

$$\frac{100 - \dfrac{100}{C_{n+1}} \cdot c_{n+1}}{100 - \dfrac{100}{C_n} \cdot c_n} = 10^{-k \cdot 10} = K \qquad \textit{Equation 2}$$

These relations show a simple way to calculate K from the quotient between the concentrations in two adjacent 10-minute samples.

In the human experiments the difficulty arises that due to lack of material, 20-minute samples must be collected. One of the sampling periods then contains the appearance times of the relevant indicator (a^N), which is unknown. It is, however, possible to make use of the fact that every sample represents the integral of the curve. We have

$$\frac{100\, c_1}{C_1} = \int_a^{20} \frac{c \cdot 100}{C} \cdot dt = \frac{100\,(20 - a)}{20} - \frac{100\,(1 - 10^{-k(20-a)})}{20\, k \ln 10} \qquad \textit{Equation 3}$$

and

$$\frac{100\, c_2}{C_2} = \int_{20}^{10} \frac{c \cdot 100}{C} \cdot dt = \frac{100 \cdot 20}{20} - \frac{100\,(10^{-k(20-a)} - 10^{-k(40-a)})}{20\, k \ln 10} \qquad \textit{Equation 4}$$

Since $(100/C_1) \cdot c_1$ and $(100/C_2) \cdot c_2$ are the experimentally found values for the first two 20-minute samples of one indicator, the equation system above may be computed by trial and error or solved graphically with the aid of a semilogarithmic paper.

With the aid of a and k the whole $(100\, c/C)/10$ curve may be constructed and used for the assessment of individual indicator flow and mean transit time at any time or time period after the in vitro experiments with constant volume and flow. The flow of indicator under a given time period is obtained from the surface between the $(100\, c/C)/10$ curve and the abscissa.

For the calculation of the mean transit time of any single indicator tm_t^N directly from the curve the frequency distribution in time of indicator particles must be known. Zierler (1) analyzed the behavior of single indicator concentrations after constant infusion and under stationary conditions. He pointed out that the increasing exponential part of the concentration-time curve fits well with the assumption of an exponential decaying time frequency distribution of all particles infused at a given short time, and has the same

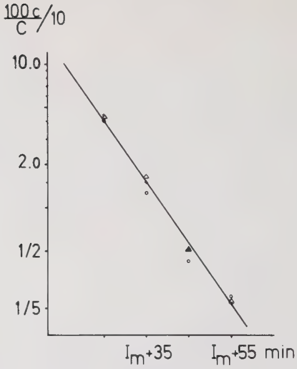

FIG. 4. Semilogarithmic slope of the (100c/C)/10 curves in Fig. 3. · Δ and + refer to different indicators.

value of k (Fig. 5). Important for the calculation is the fact that the distribution of transit times of the indicator particles infused during an infinitely short period is identical to the distribution of transit time at the outflow tract at any experimentally elapsed time (Fig. 5, lower part). According to this concept and to the definition of the mean transit time we have for a single indicator infused at a constant rate:

$$\frac{\int_o^t t\, fc\, dt}{\int_o^t fc\, dt} = \frac{\int_o^t t\, 10^{-kt}\, dt}{\int_o^t 10^{-kt}\, dt}$$

Equation 5

$$\frac{\frac{1}{k \ln 10}(1 - 10^{-kt}) - t\, 10^{-kt}}{1 - 10^{-kt}} = tm_t - a$$

t in this equation is the time after the appearance time a and fc, i.e., (100 c/C)/10) is here the flow of indicator per minute. When equilibration of in- and output of indicator has occurred tm_t is equal to tm and to Tm. Figure 6 shows the tm_t's of the curve depicted in Figure 5. The mean transit time for a 10-minute period is obtained from:

$$\frac{\int_{10(n-1)}^{10n} fc\, tm_t\, dt}{\int_{10(n-1)}^{10n} fc\, dt}$$

Equation 6

where fc is the flow of indicator per minute.

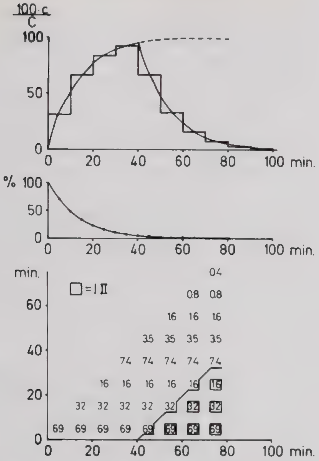

FIG. 5. *Upper.* Constant volume and flow, constant total concentration of total indicator = C. 100 units infused per 10 minutes with change of indicator every 40th minute. Theoretical $(100c/C)/10$ curve for one single indicator if the appearance time is zero and the K-value 0,0326. *Middle.* Part of the same single indicator infused during an infinite short time and mixed immediately. Percent of its initial concentration at the outflow tract after different times. *Lower.* Time-frequency distribution of concentrations. Concentration of every (during an infinitely short time) infused indicator is 100 percent at zero time of its infusion and later on as illustrated by the middle curve.

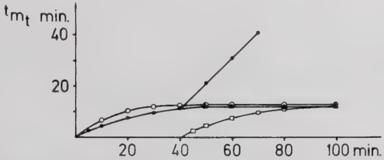

FIG. 6. tm_t calculated from the $(100c/C)/10$ curve depicted in Figure 5 according to equation (5) and equation (10). ·——· and □——□ tm_t of two successive indicators according to (5), O——O according to equation (10).

For practical purposes tm is the mean of the tm_t in the beginning and end of a period. In our experiments, where each indicator was infused into duodenum during 40 minutes Tm may be calculated from:

$$Tm + Im = \frac{c_1 t_1 + c_2 t_2 + \cdots + c_n t_n}{c_1 + c_2 + \cdots + c_n} \qquad \textit{Equation 7}$$

Thus calculated, Tm expresses the difference between the average time for the passage of an indicator at the infusion site and the average time for its passage at the sampling site. The t_1 to t_n are the times in minutes from start of the infusion to the mid-time (more correctly, the center of gravity) of each collection period (Fig. 7).

If no mixing occurs, the appearance time is equal to the mean transit time. When mixing occurs, the appearance time will be shorter and the time for the indicator to pass the sampling point longer, owing to the fact that the indicator is dispersed in a greater volume than the original. The quotient between the average time it takes the amount of one indicator to pass the site of infusion (*Im*) and the average time it takes the same amount of indicator to pass the sampling point in the "jejunum" (*Pm*) is thus a measure of the degree of mixing with contents present in the "intestine" before the indicator infusion and after its end.

Evidently *Tm* for one indicator is composed of three components *a*, *Pm*, and *Im* (Fig. 7).

$$Tm = a + (Pm - Im) \tag{8}$$

Proportional changes of volume and flow. If the volume increases proportionally to the flow into an intestinal segment, the mean transit time for indicator particles will be constant, and the flow out of the segment equal to the inflow. Under these experimental conditions $100/C_n$ is still a measure of flow per period. In the determination of mean transit time the concentrations of indicator must be weighed against the corresponding flows. Equation 7 is then transformed to

$$Tm + Im = \frac{100\left(\frac{c_1}{C_1}t_1 + \frac{c_2}{C_2}t_2 + \cdots + \frac{c_n}{C_n}t_n\right)}{100\left(\frac{c_1}{C_1} + \frac{c_2}{C_2} + \cdots + \frac{c_n}{C_n}\right)} \qquad \textit{Equation 9}$$

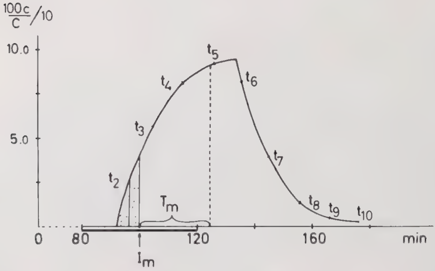

FIG. 7. Calculation of mean transit time under constant conditions from Equation 7; *Im* = mid infusion time. The dotted line is the center of gravity of the curve calculated according to Equation 7. *Tm* is the mean transit time. The time from the beginning of the infusion to the beginning of the curve is the appearance time, *a*. The time from the beginning of the curve to the dotted line is the mean passage time, *Pm*.

Disproportional changes of volume and flow. Figure 6 shows that during constant conditions tm_t calculated according to Equation 5 increases with time to be constant when equilibrium has been reached between input and output of indicator. If the inflow to a segment is kept constant but the segmental volume increases, some of the indicator particles are retained in the segment. The same occurs if the inflow is decreased and the volume is kept constant. As a result, fewer particles leave the segment per time, i.e., the flow of total indicator decreases from $100\ C/C$ to $100\ C/C - \Delta f\ C$ where $\Delta f\ C$ is the change of $f\ C$ per given period.

The decrease of indicator flow expresses a prolongation of the transit time of indicator particles. Although these originate from different infusion times, after mixing they behave approximately as originating from a given infusion time and as having a common average transit time. In Figure 8 the fact is illustrated that particles which represent the average behavior of all particles passing the sampling site at a given moment may be considered infused one *tm* earlier.

As long as *tm* is constant the indicator passes the sampling site at the same rate as it is infused. If *tm* increases linearly and all indicator particles are equally distributed per length of intestine the amount of total indicator

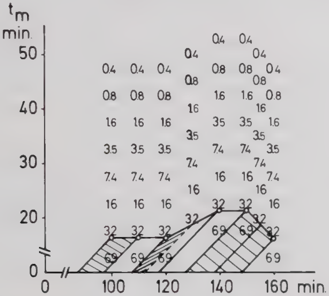

FIG. 8. The effect on flow of a change in the mean transit time in the same theoretical example as in Figure 5. Fast particles dominate the flow of indicator, wherefore the mean transit time is eccentrically located. As particles with different transit times are mixed at the sampling site and evenly distributed in the investigated segment, an increase of the mean transit time from 12.5 to 17.5 minutes between 120 and 130 minutes leads to an equal increase of the transit time of all frequencies during this period. The change of all transit times is therefore illustrated by the behavior of the mean transit time. The oblique lines in the figure show that particles with a transit time equal to tm which are infused at the time 107.5 minutes arrive at the sampling site after the mean transit time 12.5 minutes, 12.5 minutes later, i.e., at the time 120 minutes and so on.

During constant conditions the 100 units passing the sampling site during ten minutes left duodenum during a 10-minute period one tm, i.e., 12.5 minutes, earlier. When *tm* increases with 5 minutes between 120 and 130 minutes, i.e., $\Delta\ tm$ is 5 minutes, the amount passing the sampling site will be $100 - \Delta\ tm \cdot 100/10 = 100 - 5 \cdot 100/10 = 50$ units. When *tm* decreases with 5 minutes between 150 and 160 minutes the indicator amount passing the sampling site per 10-minute period will increase to $100 - (-\Delta\ \text{tm} \cdot 100/10) = 100 + 5 ° 100/10 = 150$ units.

passing the sampling site originates from a shorter time of infusion and is correspondingly less. The figure shows, that for 10-minute samples

$$\begin{aligned} \Delta f\,C &= \frac{\Delta\, tm \cdot 100}{10} = 10\,\Delta\, tm \\ f\,C &= 100 - \Delta f\,C = 100 - 10\,\Delta\, tm \end{aligned} \qquad \textit{Equation 10}$$

The equation shows, that if C is known, flow may be calculated if $\Delta\ tm$ is known and $\Delta\ tm$ if flows are known. Since further, the mean transit time tm is the sum of all tm's, the relation would give possibilities to calculate all tm's if the flows are known and vice versa.

In the beginning of the experiment the total indicator consists of one single indicator. Its concentration at the sampling site increases continuously. Since the concentrations of indicator in different parts of the investigated segment are not equal, Equation 10 is not strictly valid. Since flow of indicator and tm covary, we investigated to what extent the relation given in Equation 10 was valid also for one single continuously infused indicator before washout of initial duodeno-jejunal contents. If the deviations from correct values were small, tm_t could be calculated by consecutive addition of $\Delta\ tm$ obtained from $\Delta\ f\ C$.

Figure 6 shows the relation between tm_t at end of periods calculated according to Equation 5 and $\Delta\ tm_t$ from $\Delta\ f\ C$ according to Equation 10 during the continuous infusion of one single indicator under conditions of constant volume and flow. At the appearance of indicator both methods give identical values. Thereafter, the values obtained from $\Delta\ f\ C$ are somewhat higher. As time passes, the values converge to be identical at the time the initial duodeno-jejunal contents have been washed out.

If the infusion of the single indicator is ended after 40 minutes, the tm values from $\Delta\ f\ c$ on the descending $f\ c$ limb are lower than calculated from Equation 5. Higher tm_t values when the $f\ c$ values rise and lower when they decrease show the dominance of fast particles during early periods and of slow particles during late periods. The positive and negative deviations compensate each other when Tm is calculated as a weighted mean of the tm^N's from $f\ c^N$ according to:

$$Tm = \frac{f_1 c_1{}^N\ tm_1{}^N + f_2 c_2{}^N\ tm_2{}^N + \cdots + f_n c_n{}^N\ tm_n{}^N}{f_1\ c_1{}^N + f_2\ c_2{}^N + \cdots + f_n\ c_n{}^N} \qquad \textit{Equation 11}$$

The same compensation is obtained if tm from $\Delta\ f\ c$ is calculated as the weighted mean of tm's for two indicators which mix when the initial duodeno-jejunal contents has been washed out. We get

$$\frac{c_n{}^N \cdot tm_n{}^N + c_n{}^{N+1} \cdot tm_n{}^{N+1} + c_n{}^{N+2} \cdot tm_n{}^{N+2}}{f_n C_n} = tm_n \qquad \textit{Equation 12}$$

Calculation of tm from the slope of the (100·c/C)/10 curve; f obtained from the tm values. Although the premises for obtaining correct tm's from the indicator flow-time curves were constant volume and flow, it seemed possible that fairly good values under unstable conditions could be obtained by the use of Equations 8 and 11. Model experiments were chosen to test this possibility. The indicator flow-time curves were drawn so that the surface under the curve for each period was equal to the mean value for $100 \cdot c/C$ for this period. The a-values were calculated for each indicator according to the principle already described for constant flow and volume. The appearance times for different indicators were plotted against time and joined with straight lines. From this line, the a_n-values for single periods were read.

Within the limits of the method, K-values for different indicators in each sample were equal. From the K-values of every 10-minute sample, a complete $(100 \cdot c/C)/10$ curve was constructed according to Equations 1 and 2 and used for the determination of Pm according to Equations 8 and 11. This Pm equals pm for the period. Figures for pm per period were calculated. By adding for each period the $pm_n - 20$ and a_n values, tm_n was obtained. The tm's were similarly plotted against time and joined with straight lines. From these the tm's at the beginning and end of each period were found, their difference being $\Delta\ tm$ for the period. From $\Delta\ tm$ flow was calculated according to Equation 10. The flow values obtained with compared with those arrived at with other methods (Table I).

Primary calculation of flow, tm from the flow values. According to the premises we have in the human experiments:

$$f_1c_1^I + f_2c_2^I + f_3c_3^I + f_4c_4^I + f_5c_5^I + f_6c_6^I = 200$$

$$f_3c_3^{II} + f_4c_4^{II} + f_5c_5^{II} + f_6c_6^{II} + f_7c_7^{II} + f_8c_8^{II} = 200$$

TABLE I

In vitro experiments (301). Comparison of measured flow rates and flow rates calculated in different ways.

f_m = measured flow/20 min; $100/C$ = flow per 20 min from dilution of total indicator; f_{indir} = flow per 20 min calculated from the slope of the $100/C$ $c/10$ curve; curve; f_I = flow per 20 min calculated by means of the iteration procedure from concentrations of duodenally infused indicators; f_{PEG} = flow per 20 min calculated by means of the iteration procedure from concentrations of duodenally infused indicators and PEG; and S = standard deviation.

20 min period	f_m	$100/C$	f_{indir}	f_I	f_{PEG}
1	48.8			50.5	54.4
2	53.6			50.5	49.4
3	35.4	51.5	35.2	40.1	32.6
4	22.1	41.5	24.9	22.8	25.3
5	23.7	40.9	26.8	23.4	22.9
6	30.5	39.2	38.0	32.4	31.7
7	40.0	35.8	39.4	38.4	39.0
8	48.6	30.7	37.8	44.6	45.2
9	35.9	39.2	38.5	41.5	41.0
10	23.8	25.6	25.6	22.8	22.4
11	20.9	23.4	16.6	20.7	20.9
Sum 3–11	280.9	327.8	282.8	286.7	281.0
S of differences from f_m		13.1	5.24	3.11	2.76

for the Nth indicator

$$f_{2N-1}c^{N}_{2N-1} + f_{2N}c^{N}_{2N} + f_{2N+1}c^{N}_{2N+1} + f_{2N+2}c^{N}_{2N+2} = 200 - A$$

for the $(N + 1)$th indicator

$$f_{2N+1}\, c^{N+1}_{2N+1} + f_{2N+2}\, c_{2N+2} = 200 - B$$

where A and B may be extrapolated from the K-values of the curve. Thereby the $(100\ c/C)/10$ curve for indicator N and $N + 1$ is constructed according to Equation 1 and 2 and is completed by giving the remaining slope the same k-value. In this curve the area between the curve and the abscissa is proportional to the amount of indicator per 20 minutes. The part of the area under the extrapolated curve is A or B respectively.

We further have

$$f_1 c_1{}^P + f_2^P c_2 + \cdots f_{2N+2} c^P{}_{2N+2} = 200$$

These relations give us $N + 2$ equations and $2N + 2$ unknown flows. For all duodenally infused indicators except the first we have

$$f_n c_n = \left(\frac{100}{C_n} - \frac{\Delta f\, C_n}{C_n}\right) c_n$$

but

$$\sum \frac{100}{C_n} \cdot c_n$$

is the amount of indicator calculated from the primary flow values obtained from $100/C$. Let us call it S^N.

Thus

$$\sum \frac{\Delta f_n c_n}{C_n} = S^N - 200 \text{ or at the end of the experiment}$$

$$S^N - (200 - A) \text{ or } S^N - (200 - B).$$

This gives us further N equations or altogether $2N + 2$ equations to solve the same number of unknown flows. How this is done in practice is reported in the next chapter.

Reference

1. Zierler, K. L.: A Simplified Explanation of the Theory of Indicator-Dilution for Measurement of Fluid Flow and Volume and Other Distributive Phenomena, Bull Johns Hopkins 103:199, 1958.

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III. Multiple Indicator Dilution Method

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Experimental procedure in human experiments

Three polyvinyl tubes of different lengths are attached to each other by surgical tape and swallowed by the subjects the day before an experiment. The longest jejunal tube (Portex NT/4/90) has a rubber bag filled with a suspension of barium sulphate at its distal end and is perforated with six holes (diam: 2 mm) dispersed over the ten distal cm. The duodenal (Portex NT/2/80) and the gastric tubes (Portex NT/3/80) end at 60 and 75 cm proximal to the end of the jejunal tube.

A thin, contrast-filled, closed tube is taped between the ends of the gastric and duodenal tubes to facilitate the fluoroscopic control. Gastrographin (Schering) diluted 1:5 is injected into the jejunal tube and is immediately withdrawn after the control. When in correct position the proximal tube ends in the distal gastric antrum, the middle in the descending duodenum. The distal tube ends in the jejunum 125–135 cm from the incisor teeth. The length of the intestinal test segment corresponds to 70 cm of the tube.

Before start of the experiments the subjects are comfortably placed in semi-supine position and requested to withdraw the salivary secretion discontinuously with a suction apparatus. The gastric contents are withdrawn and samples of "basal" jejunal contents siphoned. The duodenal tube is filled with the first indicator.

A liquid test meal, marked with polyethylene glucol (PEG) is ingested during two to three minutes. Starting at the time of ingestion, three different indicators are infused into the duodenum at a constant rate (0.5 ml/min) one at a time, with change of indicator every 40th minute. The jejunal contents are siphoned during consecutive 20-minute periods, and the samples are kept on ice. Samples from the gastric antrum are withdrawn at 20-minute intervals starting at 10 minutes. The importance of a calm and pleasant atmosphere around the subject must be emphasized.

The experiments are continued until the gastric marker can no longer be detected in jejunal samples. The design of a human experiment is shown in Figure 1.

Choice of marker substances. Estimation of radiation dose

Several recent reviews (1, 2) deal with substances which according to criteria given by Hollander and Glückstein (3) can be used as markers in the gastrointestinal tract. The suitability of PEG 4000 has been convincingly shown (4). In 1963 Schütz and Reizenstein (5) demonstrated the validity of radiovitamin B_{12} as a dilution marker and in 1956 Larsson validated the use of vitamin B_{12} (6).

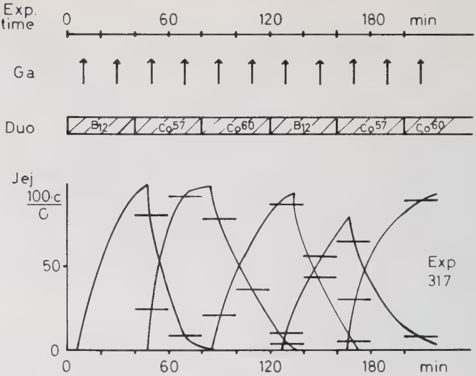

FIG. 1. Design of human experiments. In lower part the curves were drawn from the $100/C \cdot c$ values per 20 minutes for the different indicator runs in exp 317 G. Note that the areas surrounded by the curves do not amount to 200, which is the experimental evidence for a varying mean transit time.

The intrinsic factor-dependent absorption of isotope is negligible. The dose-dependent absorption 1.2 per cent is 0.0024 μC of each $B_{12}Co^{57}$ and $B_{12}Co^{60}$, provided two 40-minute runs are infused and 50 percent recovered at the sampling site. An injection of 500 μg vitamin B_{12} followed all experiments.

In comparison the absorbed dose in healthy individuals after a Schilling test exceeds 15 percent of 0.5 μC $B_{12}Co^{57}$ with a specific activity of 1 μC/g.

If the absorption can hence be regarded as insignificant, the intestine is the critical organ. In an attempt to estimate the radiation doses it was assumed that the isotope infused during 80 minutes (reduced by the recovered 50 percent) was contained in a volume of 300 ml and traveled through the small intestine in six hours and through the gross intestine in six days. The calculation for $B_{12}Co^{60}$ gave approximately 0.2 mrad and 8 mrad to the small and gross intestine respectively. Reduction of the volume to 150 ml doubles the figures. The radiation dose received from Co^{58} and Co^{57} should be respectively twice and ten times less. The fluoroscopic control of the position of the tubes adds about 20–30 mrad to these figures.

Comparative figures for an x-ray examination of the small intestine is 100 mrad and of the gross intestine 600 mrad (National Institute of Radiation Protection, Sweden).

Chemical methods

Polyethylene glycol was determined according to Hydén (7). Vitamin B_{12} was measured according to Larsson (6), and radioactivity by a well-type crystal detector (three-channel Packard model 3375).

Protein was determined with Reider's modification of the method by

Folin-Ciocalteu (8) and fat by titration of free fatty acids after saponification, acidification, and extraction. Sugar was determined by the Hagedorn method (9) and glucose by a glucose oxidase reagent (Glox, Kabi).

Lipase was measured according to Desnuelle (10), amylase according to Lagerlöf's modification of Nörby's method (11), and trypsin according to Erlanger, Kokowsky, and Cohen (12). Bilirubin was measured according to Jendrassik and Groof (13). The pH was determined with Radiometer pH meter 25. Hydrochloric acid was titrated to the pH of the test meals. Osmolarity measurements were made with a Knauer osmometer.

Methods of calculation (for explanations of symbols and definitions see Chapter II)

Jejunal flow. To facilitate computation of the jejunal fluid flows, all indicator concentrations are expressed in a common relative unit per ml. The amount of indicator infused during 20 minutes is given the magnitude 100 units, and the net amount of test meal marker, PEG, 200 units.

The calculation of the flows is based upon the fact that the sum of the products of the jejunal concentrations of one indicator multiplied by the jejunal flow volumes in which the indicator is present should amount to 200 units, i.e., to the amount infused during one duodenal indicator run. It is further based upon the assumption that the different indicators in a 20-minute sample are contained in the same volume.

The calculations are processed by a computer and involve successive repetition of two procedures.

1. Calculation of factors by which the sum of the products for the concentration of one indicator times relevant flows is 200.
2. Construction of a weighed mean from 2–4 flow values per 20-minute period.

These steps are repeated until flow values per 20-minute period multiplied by the different indicator concentrations amount to 200 units. Likewise the flows times the concentrations of test meal marker (PEG) should amount to 200 units (appendix). As starting values for the flow calculations approximate values were inserted from dilution of total indicator ($100/C_n$), where C_n is equal to the sum of individual indicator concentrations in jejunal sample n.

The first infused indicator is single in the two first jejunal samples and is diluted by intestinal contents present in the test segment at the start of the experiment. By including the jejunal concentrations of PEG into the calculations the weighing procedure could be performed during the first two periods.

The last infused indicators that have only partly passed the jejunal sampling site at the end of the experiment have to be extrapolated. The extrapolations are made from approximate flow values obtained according to the slope method (page 18).

Mean transit time. The knowledge of the jejunal flows per 20-minute period makes it possible to calculate the mean transit time (tm_n). The tm_n is defined as the average time it has taken the amount of total indicator ($f_n C_n$) passing the jejunal sampling site during period n to transit from the site of infusion to the jejunal sampling site. The change of the mean transit time ($\Delta\ tm_n$) of indicator particles during a 20-minute period is related to the amount of duodenally infused indicator which passes the jejunal sampling point during one 20-minute period ($f_n C_n$) according to:

$$f_n C_n = 100 - \frac{100 \cdot \Delta\, tm_n}{20} \qquad \textit{Equation 1}$$

where 100 units is the amount of indicator infused into duodenum each 20-minute period. From this relation it can be seen that the fluid flow f_n at the jejunal sampling site is composed of the following components:

$100/C_n$ which is the expression of the flow rate under steady state conditions and

$5\ \Delta\ tm_n/C_n$ which expresses the deviation from steady state conditions.

The change of the mean transit time of one single indicator ($\Delta\ tm_n{}^N$) during 20 minutes can be calculated according to:

$$f_n c_n{}^N = 100 - \frac{100 \cdot \Delta\, tm_n{}^N}{20} \qquad \textit{Equation 2}$$

Successive addition of the consecutive $\Delta\ tm_n{}^N$ per 20 minutes is equal to the mean transit time of those particles of N, which pass the sampling site at the end of a period:

$$tm_t{}^N = \sum_{n=1}^{t} \Delta\, tm_n{}^N \qquad \textit{Equation 3}$$

The tm_t of the total indicator may be obtained as a weighted mean from the single indicator $tm_t{}^N$ giving each $tm_t{}^N$ weight in proportion to its part of the total indicator (Fig. 2).

Another approach is to calculate the change of mean transit time of total indicator that pass the jejunal sampling site during 20 minutes from:

$$f_n C_n = 100 - \frac{100 \cdot \Delta\, tm_n}{20} \qquad \textit{Equation 4}$$

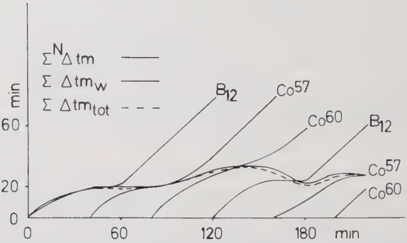

FIG. 2. $\Sigma\ \Delta\ tm^N$ (min) calculated from $f \cdot c^N$ (units/20 min) for the single indicators. $\Sigma\ \Delta\ tm$ (min) calculated from the total indicator or as a weighted mean from the 2–4 $\Sigma\Delta\ tm^N$ in each 20 minute period. The transit times used in the calculations were derived from the total indicator and are the $\Sigma\ \Delta\ tm$ (min) in the middle of each 20-minute sampling-period. Exp 317 G.

The mean transit time at the end of a period (tm_t) is then obtained from:

$$tm_t = \sum_{n=1}^{t} \Delta\, tm_n \qquad \textit{Equation 5}$$

Both methods determine the mean transit time of the total indicator at the end of a period. This latter method is easier to carry out and gives values of the same magnitude as calculation of weighted means from different tm_t^N's as demonstrated in Figure 2.

The mean transit time representative for indicator particles passing during one period, tm_n, is equal to $tm_t + \Delta\, tm_n/2$.

If the delivery of total indicator ($f_n C_n$) to the jejunal sampling site is 100 units per 20-minute period, no change of $\Delta\, tm_n$ has occurred. A change of the mean transit time leads to a delivery of more or less than 100 units per 20-minute period at the jejunal sampling site. The time to infuse an amount of indicator equal to the passed amount ($f_n C_n$) is accordingly longer or shorter than 20 minutes and is equal to ($20 - \Delta\, tm_n$) minutes (Fig. 3).

Intestinal mixing. Since the volume of the duodenal contents at the duodenal infusion site of the indicator cannot be assessed, the mixing of the intestinal contents that pass the sampling site in jejunum cannot be expressed as a volume-to-volume relationship. It is possible, however, to express the mixing as the relation between the mean passage time of a single indicator at the jejunal sampling level, Pm, to the mean passage time of the same amount of the total indicator.

This relation is obtained by calculating how much of the 100 indicator units infused into the duodenum during 20 minutes is mixed with indicator infused during earlier and later 20-minute periods during its transit through the investigated segment. If the flow rate of indicator is equal at the jejunal sampling site and the infusion site, i.e., if tm is constant, the mixing is expressed by $Pm/20$. If tm increases, the amount of total indicator which

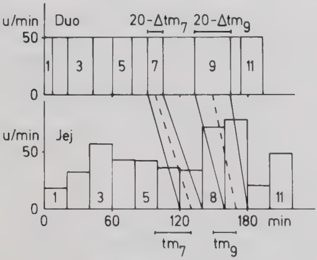

FIG. 3. The amount of total indicator which passed at the jejunal sampling level during the seventh 20-minute sampling period, $f_7 \cdot C_7$, at a rate of $f_7 \cdot C_7/20$ units/min, was infused on an average tm_7 minutes earlier during $20 - \Delta\, tm_7$ minutes, at a rate of $f_7 \cdot C_7/20 - \Delta\, tm_7 = 5$ units/min. Exp 317 G.

passes at the jejunal sampling site is less than 100 units; if *tm* decreases, more than 100 units pass at the jejunal sampling level per 20 minutes.

The mean passage time, *Pm*, for a single indicator is the average time it takes 100 units of this indicator to pass the sampling site (Chapter II, Equation 8).

The average amount of the total indicator which passes the sampling site per 20 minutes during the concomitant passage of one single indicator is determined according to:

$$\frac{c_1 f_1 C_1 f_1 + c_2 f_2 C_2 f_2 + \cdots + c_n f_n C_n f_n}{c_1 f_1 + c_2 f_2 + \cdots + c_n f_n} = B \text{ units} \qquad \textit{Equation 6}$$

If B units of total indicator pass the sampling site during 20 minutes, 100 units of the total indicator pass during $20 \cdot 100/B$ minutes.

The mixing index is accordingly determined from:

$$M = \frac{Pm}{\dfrac{20\ 100}{B}} \qquad \textit{Equation 7}$$

Calculation of gastric emptying, volumes, and secretion

Gastric emptying. The amount of test meal marker which passes the jejunal sampling site during the n^{th} 20-minute period, has traveled through the intestine at the same velocity as the indicator infused into duodenum and entered the test segment tm_n minutes earlier during $20 - \Delta\, tm_n$ minutes. Knowing the amounts of PEG which pass the jejunal sampling site during consecutive 20-minute periods, the times when they left the stomach and further the times during which they left the stomach, the gastric emptying of PEG can be determined. The emptying of gastric contents, i.e., gastric secretion and test meal marker, can thus be determined since antral concentrations of PEG are known. The gastric emptying rate may be expressed per minute (by dividing the jejunal portion of PEG with the emptying time) or be adjusted to 20-minute periods to facilitate comparisons between experiments and meals.

Gastric volumes and secretion. By continuous subtraction of the emptied 20-minute portions of PEG (and of the amounts withdrawn in gastric samples) the PEG remaining in the stomach is determined. The remaining PEG divided by the concentration of PEG is equal to the volume of the gastric contents. Gastric secretion (S) is estimated according to:

$$S = V_t - V_o + E_{o-t} + P \qquad \textit{Equation 8}$$

V_o volume at time 0 (ml)
V_t volume at time t (ml)
E_{o-t} evacuated volume during time o to t (ml)
P volume of gastric sample (ml)

Gastric concentrations of PEG in regurgitated samples were corrected for the duodenal reflux as described below.

Duodenal reflux. The concentrations of PEG in gastric samples, which were contaminated with bile, have to be corrected for the dilution from regurgitated duodenal juice. This can be done from the acidity values of the withdrawn samples (H_f), provided that the concentration of hydrochloric acid in the gastric secretion (H_s) and the concentration of bicarbonate in the mixed duodenal juice are known. H_s is constructed for each experiment as a weighted mean of the acidity values of the gastric juice in not regurgitated samples. The concentration of bicarbonate in the mixed duodenal secretion is assumed to be 70 mEq/1.

The amount of hydrochloric acid in one ml of antral contents is equal to the amount in the gastric juice minus the amount neutralized by duodenal reflux.

$$(1 - q)\,1 \cdot H_s - (1 - F)(1 - q) \cdot 1 \cdot 0.070 = H_f \cdot 1$$

$$= \frac{0.07\,(1 - q) + H_f}{(H_s + 0.07)(1 - q)} \qquad \textit{Equation 9}$$

where

H_f = hydrochloric acid concentration in a gastric sample (mE/ml)
$q \cdot 1$ = volume of meal in one ml of a gastric sample (ml)
$(1 - q) \cdot 1$ = volume of secretion and regurgitation in one ml of a gastric sample (ml)
F = a factor denoting the part of $(1 - q) \cdot 1$ which is gastric juice
$F(1 - q) \cdot 1$ = volume of gastric juice in one ml of a gastric sample (ml)
$(1 - F)(1 - q) \cdot 1$ = volume of duodenal juice in one ml of a gastric sample (ml)

The concentration of PEG in a contaminated gastric sample multiplied by

$$\frac{1}{q + (1 - q) \cdot F} \qquad \textit{Equation 10}$$

is equal to the concentration of PEG in the antral contents not containing duodenal reflux.

The volumes of the duodenal reflux may be estimated indirectly as the difference between the emptied volumes obtained with uncorrected and corrected concentrations of PEG.

Output of bile and pancreatic enzymes. The amount of bilirubin and of pancreatic enzymes passing the jejunal sampling site each 20-minute period left the duodenal level tm_n minutes earlier. The outputs of bilirubin and of pancreatic enzymes are calculated according to the method described for the gastric evacuation.

Intestinal absorption.

c_{sg_n}/c_{pg_n}	ratio between gastric concentrations of nutrient s and PEG corresponding to the jejunal sampling period n, i.e. the concentrations prevailing in gastric antrum tm_n minutes earlier.
$c_{sg_n}/c_{pg_n} \cdot c_{pj_n} \cdot f_n$	L_n, the intestinal load of nutrient s (mg) corresponding

	to the jejunal period n, i.e. the amount of s should pass the sampling site during 20 minutes if the absorption was nil. The load left the stomach tm_n minutes earlier during $20 - \Delta\, tm_n$ minutes.
$c_{sJ_n} \cdot f_n$	P_n amount of nutrient s (mg) which passed the jejunal sampling level during 20 minute period n.
$L_n - P_n$	A_n amount of nutrient s which was absorbed from L_n during transit of the proximal 70 cm of the intestine (mg/70 cm/tm min).
A_n/L_n	part of L_n absorbed during transit of the proximal 70 cm of the intestine.
A_n/tm_n	average rate of absorption from L_n during transit (mg/70 cm/min).
$L_n/20 - \Delta\, tm_n$	rate of gastric emptying of L_n (mg/min).

Validity of the method

To test the validity of the method for calculation of the intestinal fluid flows and gastric emptying experiments were performed in an in vitro model of stomach and intestine (Chapter II, Fig. 2).

A beaker containing a test solution of polyethylene glycol (PEG) acted as a stomach. By an attached pump the inflow rate to a 75 cm long collodium tube could be regulated. By a sinusoid arrangement of "the intestine" and a rocking devise the "intestinal" contents were mixed. Three different indicators were infused successively at a constant rate 5 cm distal to the inflow site. At the exit of the "intestine," timed collections of fluid were made (10-minute sampling periods).

During the experiments variations of the "intestinal" volumes were induced by changing the inflow rate from the "stomach" and by elevating the exit site of the test segment. The gastric contents were continuously diluted.

The outflow rates from the tube and the "gastric emptying" were calculated and compared to the collected fluid volumes and used pump velocities.

The design of and results from three experiments are given in Figure 4a–c. A good agreement was found between calculated and measured flow rates (ml/10 min) even under conditions with rapidly induced changes of the "intestinal" volumes (Exp. 335). The calculated "gastric emptying" of PEG (units/10 min) showed satisfactory correspondence to values obtained from pump velocities, the S.D. of differences amounting to 1.88, 2.73 and 3.73 units per 10 minutes in exp 301, 304 and 335 respectively (Fig. 5).

The indicator flow-time curves recorded in experiments with the in vitro model show a close correspondence to the curves obtained in experiments with human subjects.

Reproducibility of method

Duplicate experiments were performed in three subjects, who on two occasions were examined after ingestion of a glucose-containing composite

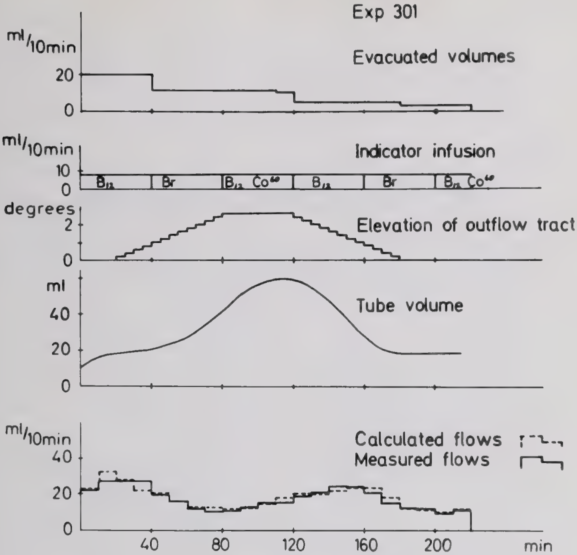

FIG. 4a–c. Design of in vitro experiments. Comparison of measured and computed fluid volumes at the exit from the tube acting as intestine (ml/10 min).

meal (G-meal). No systematic differences were found between the first and second experiment.

Comparisons were made between the variance within the three subjects and the variance between the seven subjects reported in this series (Table I).

Although not significantly, the S.D., for the mean amounts of PEG, emptied during the first hour was less within than between subjects. Linear regressions were calculated from 5–7 values in each experiment between time and in PEG (percent) retained in the stomach. The b-values were pooled in duplicate experiments and in the group. No difference was found between a pooled S.E. (b) within and between subjects.

Methodologic remarks

To simplify calculations, the jejunal sampling periods should be even divisors of the duodenal infusion periods. The lengths of the sampling periods are determined by the necessity of obtaining samples from each period. The siphoned volumes should be large enough to allow duplicate analyses of all indicators and preferably duplicate analyses of the different test meal components, pancreatic enzymes, and bilirubin. We cannot at present define

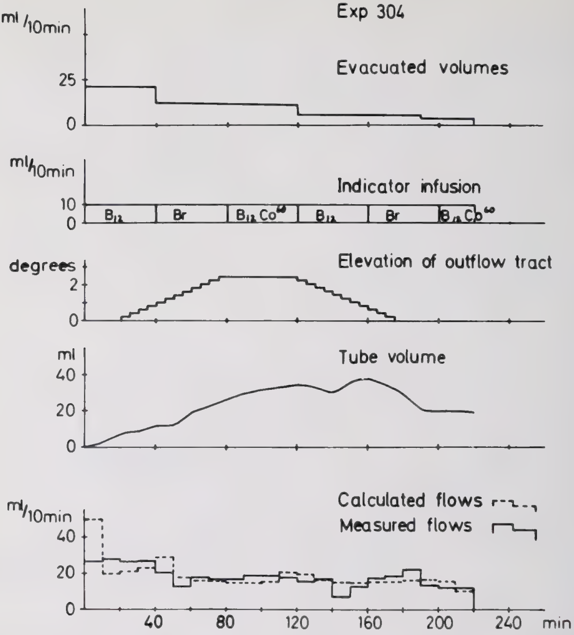

FIG. 4b.

an upper limit for the withdrawn jejunal contents, but it is to be suspected that gastric regulatory mechanisms might be interfered with if the spreading of the emptied contents over a large mucosal area is interrupted. When the first emptied portions are large, travel at a high velocity in the intestine, are relatively poorly absorbed, and are contained in large flow volumes, the risk of withdrawing material with potential regulatory effect should be considerable. Experimental observations during the compilation of this method indicate a remarkably rapid gastric emptying, when large amounts of the meal are withdrawn through a tube, ending at the ligament of Treitz.

In the present series the sampled volumes were an even percentage of the calculated jejunal fluid flows (Table II). As a result of the high percentage absorption at the investigated level, the withdrawn amounts of the food components were small.

To ensure that the siphoned samples correspond in time to the sampling periods, correction for the "dead space" of the sampling tube (8 ml) is made at

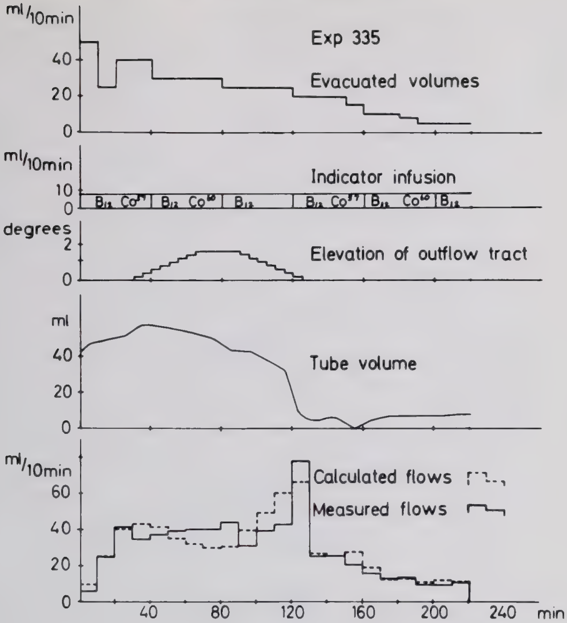

FIG. 4 c.

the end of each 20-minute period, with 8 ml if the tube was filled, or if not, in proportion to the fluid volume in the visible part of the tube.

The sampling volumes are "functional" volumes, since they represent a net result of propulsive and retropulsive movements of fluid at the level of sampling. The same applies to the calculated fluid flows. Likewise, the sampling site is functionally rather than anatomically defined, as in all intubation techniques. Telescoping of the tube may occur during the experiments, although not supported by the experimental data, and, according to Hirsch, Ahrens, and Blankenhorn (14), telescoping should be moderate with tubes of the used diameter.

The test segment, or rather the mean transit time through the segment, must be long enough to allow mixing of the different indicators. On the other hand, should it be too long, the different runs of one indicator are mixed. In the experiments here reported only minute amounts of an indicator were left in the segment when its next infusion was started after 80 minutes. Separation

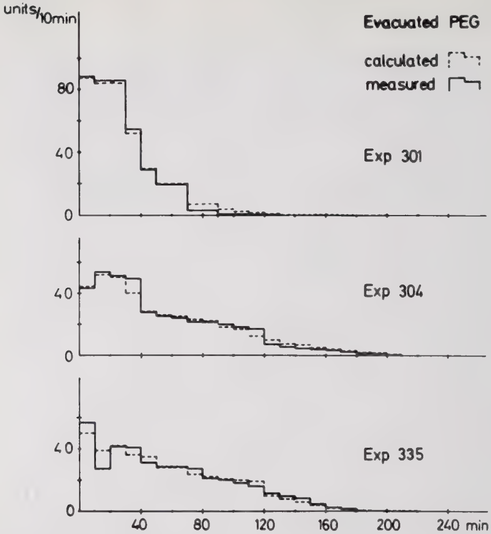

FIG. 5. Comparison of used pump velocities and calculated "gastric emptying" of PEG (units/10 min) in model experiments.

TABLE I

Reproducibility of method. Mean ± S.D.$_w$ (within subjects) for the amount of PEG (unit) emptied within the first hour after intake of the G-meal in three duplicate experiments compared to Mean S.D.$_i$ between (e.g., inter) seven subjects after the same meal.

Linear regressions between time and the L_n PEG (percent) remaining in the stomach were calculated; *b*-values were pooled in duplicate experiments and in the group; and the pooled S.E. (*b*) compared within and between subjects.

Exp no	1st hour units	ln *b*	pooled ln S.E. (b)
371	154.1	−.747	±.024
374	146.4	−.512	
375	168.4	−.868	±.026
376	176.3	−1.002	
373	142.2	−.569	±.013
385	159.1	−.553	
Mean	157.9		
S.D.$_w$	±8.4		±.022
G-meals			
Mean	124.5	−.443	
S.D.$_i$	±18.3		±.023

TABLE II

Sampled hourly volumes of jejunal juice in percent of the calculated hourly fluid flows. Means and S.E.M.

		1st	2nd	3rd hour
NG-meals	Mean	41	39 ± 6	33 ± 7
	S.E.M.	± 7		
G-meals	Mean	60	51 ± 10	45 ± 9
	S.E.M.	± 8		
GP-meals	Mean	37	35 ± 13	40 ± 12
	S.E.M.	± 9		
15%	Mean	18	29 ± 9	
	S.E.M.	± 5		

was made, when necessary, according to the rate of decline of simultaneously present indicators.

The infusion of physiological saline in the descending duodenum adds a volume of 10 ml/20 minutes to the duodenal contents. The effect on the gastric emptying rate should be negligible (15) and it is unlikely that the duodenal events are affected. Borgström et al reported a three- to fivefold dilution of the G-meal in the duodenum (16) and a fourfold dilution of a milk and doughnut meal was recorded by Fordtran and Locklear (17). An infused volume of 90 ml in three hours should represent 6 to 10 percent of the net volume in which it is contained in the duodenum and considerably less if compared to the total duodenal water exchange.

From what has been discussed it follows that the lengths of the test segments, duration of the single indicator infusions and of the sampling periods are not fixed entities, but should be defined for each test meal according to the gastric emptying rate, the mean transit times, and the absorbability of its components.

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Appendix

Computer Program for Calculation of Jejunal Flow

$$f_n^{\,k} = \frac{100}{(a_n + b_n + c_n)^{k+1}} \prod_{k=1}^{s} \left(\frac{200}{A_k} \cdot a_n + \frac{200}{B_k} \cdot b_n + \frac{200}{C_k} \cdot c_n \right)$$

$k = 1, 2, \cdots$, number of iterations

$n = 1, 2, \cdots$, number of periods, during which the experiment lasts

$f_n^{\,k}$ flow in period n after k iterations (ml)

a_n concentration of indicator A in period n (units/ml)

b_n concentration of indicator B in period n (units/ml)

c_n concentration of indicator C in period n (units/ml)

$$A_k = \sum_{n=r}^{r+p} f_n^{\,k-1}\, a_n$$

$$B_k = \sum_{n=r+2}^{r+2+u} f_n^{\,k-1}\, b_n$$

$$C_k = \sum_{n=r+4}^{r+4+v} f_n^{\,k-1}\, c_n$$

$r, r + 2, r + 4$ periods in which infusion of indicators A, B, C, are started

p, u, v number of periods during which indicator A, B, C appears at outflow tract.

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IV. Studies of Four Different Meals

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Test meals

It is reasonable to believe that interactions exist between the stimulatory and inhibitory effects of different food components on gastric and intestinal functions. A physiologic approach to the study of the effects of individual food components is to compare changes of meal composition relative to a standard meal. One of the meals in the present study is essentially the same as that used by Borgström, Dahlqvist, Lundh, and Sjövall in their classic study on intestinal absorption (1). It contains protein, fat and carbohydrates in the same proportions as in an ordinary Swedish diet and is in the following referred to as the G-meal. The G-meal was changed by exclusion of the glucose (NG-meal), by doubling the protein (GP-meal), and by exclusion of fat, protein, and lactose (15 percent glucose solution). This enabled us to study the influence of glucose by comparing the results from the NG and G-meal, of protein by comparing the GP and G-meal, and of protein and fat by comparing the 15 percent glucose solution and the G-meal.

The composition of the meals and some physical properties are given in Table I. The energy content of the meals was determined according to the figures by Rubner (2), i.e., for lactose 3.88, for glucose 4.00, for milk protein 3.96, and for corn oil 9.38 kcal/g.

Material

The number of investigated subjects is given in Table II together with sex, age, body weight, and Broca's index. Because of lack of material for the analyses, all variables could not be assessed in all subjects; the number of included experiments is therefore reported in the separate chapters. Duplicate experiments were performed in seven subjects with the NG and G-meal.

Two further experiments with the 15 percent glucose solution are included in the chapter on pancreatic enzymes. Since only average hourly figures for the

TABLE I
Composition of the Test Meals (g/100 ml.)

	NG	G	GP	15%
Lactose	5.1	5.1	5.1	—
Glucose	—	10.0	10.0	15.0
Corn oil	5.9	5.9	5.9	—
Milk protein	3.6	3.6	7.2	—
PEG	3.0	3.0	3.0	3.0
kcal/100 ml	89	129	144	60
pH	6.6	6.6	6.6	4.6
mosm/l	340	950	1120	965

TABLE II

*Material. * Duplicate experiments were performed in seven subjects with the NG and G-meal.*
*** Broca's index body weight/body length – 100*

		NG	G	GP	15 %
Investigated subjects nr		7*)	7*)	7	7
Sex	male	5		7	5
	female	2			2
Age	mean	25		25	25
	range	21–33		21–32	20–28
Body w.	mean	69.1		70.1	65.4
	S.D.	8.4		9.4	5.9
Index**	mean	0.93		0.88	0.90
	range	0.78–1.03		0.75–1.00	0.83–0.95

pancreatic secretion were available from the first two twin experiments with the NG and G-meal, five experiments with the NG-meal and eight with the G-meal are included in this chapter (five plus three experiments from the reproducibility test).

The distributions of body weights and ages were essentially the same in all samples of subjects. The subjects were medical students and nurses who gave informed consent to the investigation. All were healthy at the time of the examination and none had any previous history of gastrointestinal disease or symptoms.

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V. Quantitative and Qualitative Aspects of the Gastric Secretory Response after Meals of Different Composition

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Gastric contents

The pH, acidity, and osmolarity of the antral content. The pH in withdrawn antral samples declined gradually with time after the composite meals, faster after the NG-meal, and reached a value of 2 at 1 to 3 hours after feeding the meal (Table I). Following ingestion of the 15 percent glucose solution at pH of 2 was recorded within half an hour.

The acidity of the gastric contents (Table I) increased slowly throughout the experiments.

After about 2 hours the osmolar concentration of the antral contents was isotonic, i.e., around 300 mosm/l, except in the NG-experiments where the osmolarity of the test meal only slightly exceeded that of serum.

Acid concentration in gastric secretion. In nonregurgitated samples after the NG-meals the concentration of hydrochloric acid in the secreted gastric juice (H_s) was on an average 112 ± 2.1 mEq/l. The acidity of the gastric juice was constant, i.e., no correlation existed between H_s and $1 - q$ (q is the volume of the test meal in 1 ml of the gastric contents and $1 - q$ is hence the volume of the secreted gastric juice in one ml of the gastric contents).

After the G-meals the acidity of the gastric juice increased during the experiment and was positively correlated to $1 - q$ according to the linear regression: $H_s = 72.5$ mEq/l + $(1 - q)$ 37.5 mEq/l (r + 0.47, n:36 all nonregurgitated samples with values of $1 - q$ exceeding 0.3 included). The average concentration of hydrochloric acid in the secretion was 99.7 ± 2.3 mEq/l at an average $1 - q$ of 0.76.

In three single experiments, where the number of nonregurgitated periods was sufficient to test individual regressions, correlation coefficients of +0.94, +0.89, and +0.86 were obtained (Experiment numbers 314 G, 317 G, and 336 G, respectively).

In seven experiments with the GP-meal the regression could be tested in five. Significant correlations were obtained in four of these (r +0.92, +0.93, +0.86, and +0.59 in Experiment numbers 384 GP, 382 GP, 383 GP, and 388 GP, respectively). For all nonregurgitated samples with $1 - q$ higher than 0.3 we got the following regression: $H_s = 49.4$ mEq/l + $(1 - q)$. 81.0 mEq/l (r + 0.40, n:40). The average value was 109.4 ± 5.2 mEq/l at an average $1 - q$ of 0.74.

In vitro experiments were performed to analyze whether an addition of acid radicals from digested meal protein could account for the higher acidity of the secreted juice towards later periods. Equal volumes of the G-meal (pH 6.6)

TABLE I

Acidity (mEq/l) and pH in antral samples after various meals. Mean ± S.E.M.

Time after meal intake (min)		10	30	50	70	90	110	130	150	170	190	210
pH												
NG	Mean	5.63	4.67	3.17	2.50	1.78	1.85	1.80	1.67	1.65	1.60	
	S.E.M. ±	0.54	0.32	0.58	0.34	0.27	0.28	0.26	0.26	0.22	0.29	
G	mean	6.36	5.24	4.21	3.86	3.70	2.85	2.35	3.11	2.82	2.79	2.98
	S.E.M. ±	0.17	0.35	0.14	0.18	0.48	0.28	0.33	0.94	0.60	0.56	1.09
GP	mean	5.5	4.5	3.4	2.9	2.8	2.4	2.3	2.0	1.8	1.6	1.3
	S.E.M. ±	0.16	0.51	0.67	0.57	0.62	0.51	0.41	0.91	0.29	0.71	0.66
15 %	mean	2.50	2.01	1.85	1.79	1.68	1.59	1.51	1.67			
	S.E.M. ±	0.19	0.19	0.17	0.20	0.18	0.15	0.17	0.35			
Acidity												
NG	mean	16.2	27.5	52.9	70.7	85.4	77.9	91.5	94.4	88.0	77.2	90.1
	S.E.M. ±	3.2	4.5	9.0	8.1	7.9	10.0	7.9	9.9	5.0	7.6	12.5
G	mean	7.6	16.4	23.2	29.2	35.4	47.3	56.9	61.3	70.5	76.9	88.0
	S.E.M. ±	2.8	3.9	4.7	4.4	5.6	4.0	9.9	11.7	10.5	13.6	13.6
GP	mean	2.6	24.8	41.9	56.7	61.5	71.3	78.1	85.6	95.1	97.1	99.5
	S.E.M. ±	3.6	7.3	11.5	11.0	13.3	12.8	11.6	14.2	14.2	14.2	10.0
15 %	mean	7.2	15.5	24.8	33.1	48.8	62.4	71.5	70.0			
	S.E.M. ±	4.1	5.1	7.5	10.0	11.2	27.9	11.8	16.0			

and gastric juice (pH 1.25) were incubated at 37°C. No significant change in the pH or acidity could be detected during three hours following incubation.

After the glucose solution the acidity of the gastric secretion was of constant and considerably lower than recorded after the composite meals. The average acidity in 28 gastric samples were 83.6 ± 4.7 mEq/l at an average $1 - q$ of 0.72, which is significantly lower than obtained after the composite meals (15% vs NG and GP $p < 0.001$, vs G $p < 0.005$).

Regurgitation. The number of stained samples and the regurgitated volumes were highest after the G-meal. Of all gastric samples, bile staining occurred in 49 percent, compared to 27 percent after the NG-meal, 30 percent after the GP-meal, and 14 percent after the glucose solution. In five experiments with the G-meal the average regurgitated volume was 88 ml (range 14–192 ml).

In calculating the intragastric variables, the concentrations of PEG in regurgitated samples have been corrected for dilution from duodenal reflux by the earlier described dilution factor (Chapter III, Equation 10).

Concentrations of test meal marker (PEG) in the gastric antrum. In consecutive samples from the distal gastric antrum the concentrations of PEG declined regularly. Starting at 20 to 80 minutes after meal intake, the concentration-time curves followed a semilogarithmically linear decline after all meals. Regressions between time and the logarithm of the PEG concentrations were calculated for each experiment, including values from at least five successive periods after the composite meals and three successive periods after the glucose solution (Table II).

Distribution of PEG in the gastric contents. Experiments were performed in healthy subjects to examine the distribution of PEG in the gastric contents

TABLE II

Fractional decline of the gastric concentrations of PEG in consequtive samples from the gastric antrum (percent/20 min). Means ± S.E.M. of the individual regressions. Correlation coefficients (r) *are given with means and ranges*

	r mean range	Fractional decline mean ± S.E.M.
NG	0.99 0.98–1.00	34.0 ± 4.5
G	0.97 0.91–1.00	25.1 ± 3.2
GP	0.97 0.94–1.00	23.7 ± 3.9
15 %	0.98 0.97–0.99	22.6 ± 2.0

after the G-meal. Double-lumen tubes were placed with the distal tube ending in the antrum and the proximal at a distance of 8, 11, or 14 cm. The mean ratios between the concentrations of PEG in simultaneously withdrawn samples from the distal and proximal tubes were

distance between tubes (cm)	*n*	ratio between concentrations distal/prox.	difference between concentrations distal/prox.
8	16	1.03 ± 0.037	n.s.
11	40	1.42 ± 0.087	$p < 0.001$
14	17	1.47 ± 0.118	$p < 0.005$

The results suggest an uneven distribution of PEG in the water phase of the gastric contents, with lower concentrations in the upper parts of the stomach. The proportionally larger variations of the quotients when the tubes were widely apart further points to an irregularly declining concentration curve in upper compared to lower parts of the gastric contents.

Gastric volumes and secretion. Elimination of PEG

Early after feeding of either meal the amounts of PEG decrease faster than the concentrations, indicating an early domination of the emptying over the secretion. From the second hour and on, the rate of secretion and rate of emptying usually approached each other, resulting in a constant or slowly decreasing gastric volumes, as opposed to the early rapid decline (Fig. 1 a–d).

The elimination of PEG is faster after the NG-meal than after any other meal. After one hour only 14.4 ± 1.8 percent remains in the stomach as opposed to the 37.8 ± 3.3 percent after the G-meal ($p < 0.001$). After both the GP-meal and the sugar solution, the remaining PEG approaches the figure for the G-meal (GP 38.3 ± 5.2 percent, 15% glucose 29.5 ± 4.2 percent).

The early elimination of a large portion of PEG from the NG-meal was followed by a rapid decline in the gastric volumes, the average volume one hour after meal ingestion amounting to 103 ± 10 ml. In comparison the slower elimination of the G-meal resulted in an average volume of 173 ± 12 ml (p <

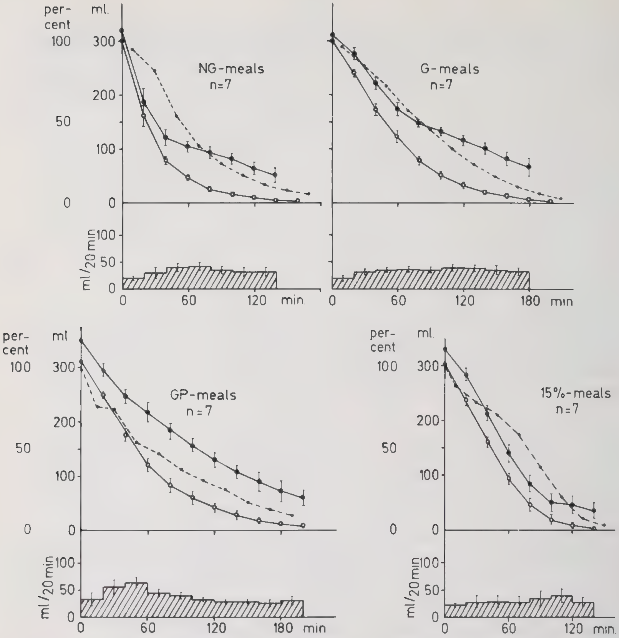

FIG. 1 a, b, c, d. Mean ± S.E.M. for gastric concentration of and elimination of PEG, gastric volumes and secretion after different meals. ○——○ PEG remaining in stomach (percent of the amount in the test meal). - - - gastric concentrations of PEG (percent of concentration in test meal). ●——● gastric volumes (ml). ▨ gastric secretion (ml/20 min).

0.01). The gastric volume at 60 minutes after feeding was higher after the GP-meal 215 ± 21 ml, whereas after the glucose solution it amounted to 140 ± 16 ml.

Since the first hourly elimination of PEG was equal after the G and GP-meal the volume differences are likely to result from differences in the gastric secretion rate.

A higher secreted volume was also recorded during the first hour after ingestion of the GP-meal compared to the G-meal (156 ± 35 ml and 91–10 ml respectively). No difference in secretion rate was found between the G-meal and the NG-meal (89 ± 17 ml during the first hour) and the volume was almost as high after the 15 percent glucose solution (79 ± 15 ml).

During the second hour the small differences in the secretion rates persisted, the volume amounting to 126 ± 14 ml (GP), 116 ± 11 ml (G), 109 ± 17 ml (NG) and 92 ± 23 ml (15%). At the end of the second hour the emptying of the NG-meal and 15 percent glucose solution are almost completed, whereas after the two other meals the continued secretion proceeded at the same constant rate for one more hour.

Comparing the meals as to the volumes secreted from start of the experiments until 97.5 percent of the PEG had been eliminated showed the total secretion to be highest after the GP-meal (371 ± 30 ml) and almost as high after the G-meal (316 ± 29 ml). It was lower after the NG-meal, 216 ± 26 ml (NG vs G $p < 0.05$) and lowest after the glucose solution 165 ± 24 ml (15% vs GP $p < 0.001$, vs G $p < 0.005$).

A fair estimation of the secreted hydrochloric acid is derived by multiplying the total secreted volumes in each experiment with the average acidity in non-regurgitated samples. After the NG-meal a total of 23.7 ± 2.8 mEq was secreted. Corresponding figures for the glucose containing meals were for the G-meal 31.6 ± 3.0 mEq for the GP-meal 40.5 ± 3.4 mEq and for the glucose solution 13.8 ± 3.2 mEq.

Discussion

Regurgitation. Regurgitation was more apt to occur after the composite meals. Since the load to the duodenum whether expressed as calories or osmoles is higher after such meals, the secretory stimulus should be stronger, resulting in a larger volume of the duodenal contents. The relatively small regurgitated volumes agree with the report from Gustke et al (1) who observed a decreased reflux of an intraduodenally infused indicator during gastric emptying of a test meal, compared to that recorded at low or very high duodenal perfusion rates in the absence of concomitant emptying.

The intraduodenally infused indicators never appeared in the stomach, indicating that the duodenal reflux never originated from parts lower than the mid-duodenum.

Gastric mixing. A complete and instantaneous mixing between a marker substance and the gastric contents has been regarded as a prerequisite for the determination of gastric volumes and secretion.

After ingestion of a meal, the proceeding gastric secretion mixes unevenly with the gastric contents, the mixing being influenced by factors like gastric volumes, secretion rate, and motility. It should however be recognized that if an evenly declining concentration curve of PEG can be obtained from some part of the stomach, the only error inherent in the calculation of gastric volumes and secretion could arise from a nonrepresentative timing of the concentrations. (In this statement it is assumed that the concentrations of PEG in regurgitated samples have been corrected by a dilution factor according to earlier description.)

Our assumption of an even mixing in the gastric antrum is supported by the high correlation coefficients obtained in individual experiments for a semilogarithmically linear decline of the antral concentrations of PEG against time. Further support was provided by human experiments with double-lumen tubes placed with their distal part in the gastric antrum and their proximal part at a varying distance. The concentrations of PEG in consecutive samples from different locations indicate a more even mixing in the distal compared to the proximal parts of the stomach.

Results from the same experiments also suggest that the dilution of the antral contents by gastric secretion is delayed compared to those in more proximal parts of the stomach. Such a delay is most likely to occur when gastric volumes are high, i.e., shortly after meal ingestion. It should lead to an underestimation of the early gastric and secretory volumes which, however, should be counterbalanced by an overestimation in the ensuing periods. If a delay persists after the initial hour, the calculated volumes should be too low, but the secretion rate unaffected. It was controlled that "antedating" of the concentrations of PEG by as much as 20 minutes had limited effect on the results and did not influence on the comparisons between the meals.

Gastric secretion. The higher the nutritional content of the test meal, the higher the volume of secreted gastric juice (i.e., from meal intake until 97.5 percent PEG had left the stomach). The differences recorded between the NG and G-meal and between the 15 percent glucose solution and G-meal can altogether be attributed to the slower emptying of the G-meal and hence to a longer lasting stimulation of the gastric secretion after this meal compared to the other. Only after the GP-meal did a somewhat higher gastric secretion rate contribute to the higher secreted volume produced during the same time as that after the G-meal.

The gastric secretion rate, which was moderate after all meals, proceeded at a constant rate throughout the entire experiments and was hence not affected by the decreasing pH of the antral contents.

Differences between the meals existed not only as to quantity but also as to quality of the secreted juice.

In contrast to the constant acidity in the secreted gastric juice recorded after the NG-meal (112.6 ± 2.1 mEq/1) the acidity increased successively after both the G and GP-meals. A linear relation was found between the acid concentration in the secretion and $1 - q$ (ml gastric secretion in 1 ml gastric contents). Since these meals remain for a considerable time in the stomach, it was suspected that amino acids from meal protein degradation contributed to the higher acidity towards the end of the experiments. This theory, however, could not be supported by in vitro experiments in which the G-meal was incubated with gastric juice at 37°C. Nor could it explain why the acidity of the gastric secretion after the G and GP-meals was lower than 100 mEq/1 when 1 q was below 0.7, and by then approached the low constant acidity recorded in the gastric secretion after feeding a glucose solution (83.6 ± 4.7 mEq/1).

From the work by Ihre (2) it is known that the acidity increases at an increasing gastric secretion rate. The differences in secretion rates recorded between our meals were too small, however, to explain a variable acidity.

If we assume that the acid secreted from the parietal cells is of a constant acidity, at least two interpretations of our findings are possible:

1. By action of the hypertonic test meals water is osmotically drawn from

the stomach wall, or

2. The emptied glucose triggers mechanisms by which the nonacid gastric secretion is stimulated.

If water is osmotically drawn from the stomach wall, we could hardly explain why, after feeding a glucose solution, both the secretion rate and the acidity of the secretion are kept constant throughout the experiments, since due to the continuous dilution, the osmolar concentration of the gastric contents is continuously decreasing.

Duodenal instillation of a hypertonic solution of glucose is a known stimulus to the alkaline duodenal and pancreatic secretions. Whether or not the nonacid gastric secretion is stimulated by emptied glucose remains to be proven. Such a mechanism should provide an acceptable explanation of our finding of a low constant acidity in the gastric secretion after feeding a glucose solution and a low acidity in the gastric juice secreted during the first hours after intake of glucose-containing composite meals.

After the composite meals the output of hydrochloric acid was about half the response obtained after maximal stimulation wih pentagastrin. Whether this modest acid output is due to feeble stimulation or strong inhibition cannot be concluded, but it supports the old clinical observation that milk meals of limited volumes are well tolerated by persons with a high secretion of acid.

The relation between gastric emptying and secretion. An assumption of single fractional rates for the decline of the concentrations and the amount of PEG was tested for its effect on the calculated gastric secretion, emptying and volumes. Although the experimental data agreed with this assumption for a considerable time in each experiment, it could not be used to describe the entire course of events because deviations from single exponential rates were present both at the beginning and end of the experiments.

The assumption of single fractional rates is, however, useful in an analysis of the relations between the two physiological processes of which the curves are the result, i.e., the gastric secretion rate (ml per minute) and the rate of gastric emptying (ml per minute).

Relations between gastric concentrations and amounts of PEG to gastric secretion, emptying and volumes:

C_{pgn}	gastric concentration of PEG at the end of period n (units/ml)
P_n	amount of gastric PEG at the end of period n (units)
E_n	emptied volume during period n (ml)
S_n	gastric secretion during period n (ml)
V_n	gastric volume at the end of period n (ml)
n	20-minute period number $(1, 2, \cdots n)$

Relations used in the calculations:

$$V_n = \frac{P_n}{C_{pg_n}} \qquad \textit{Equation 1}$$

$$E_{n+1} = \frac{P_n - P_{n+1}}{\frac{C_{pg_n} + C_{pg_{n+1}}}{2}} \qquad \textit{Equation 2}$$

$$S_{n+1} = V_{n+1} - V_n + E_{n+1} \qquad \textit{Equation 3}$$

If gastric concentrations and amounts of PEG are declining exponentially, the following relations are obtained:

$$\frac{P_{n+1}}{P_n} = k_1 \qquad \textit{Equation 4}$$

$$\frac{C_{pg_{n+1}}}{C_{pg_n}} = k_2 \qquad \textit{Equation 5}$$

where k_1 and k_2 are constants.
According to Equations 1, 4, and 5:

$$\frac{V_{n+1}}{V_n} = \frac{\dfrac{P_{n+1}}{C_{pg_{n+1}}}}{\dfrac{P_n}{C_{pg_n}}} = \frac{k_1}{k_2} \qquad \textit{Equation 6}$$

According to Equations 2, 4, and 5:

$$E_{n+1} = V_n \frac{2(1 - k_1)}{(1 - k_2)} = k_1 \cdot V_n \qquad \textit{Equation 7}$$

where k_1 is a constant.
According to (3), (6) and (7):

$$S_{n+1} = V_n \left(\frac{k_1}{k_2} - 1 + \frac{2(1 - k_1)}{(1 - k_2)}\right) = (k_2 + k_1)\, V_n \qquad \textit{Equation 8}$$

where k_2 is a constant.

From Equation 6, it is seen that the gastric volume (V_n) follows an exponential course. Emptied volumes (E_n) and gastric secretion (S_n) can be expressed as constants times V_n (Equations 7 and 8) and also behave exponentially.

In all circumstances here described the relation between the emptying rate and secretion rate can always be expressed in terms of a relation between constants which in turn are determined by the rate constants for the fractional decline of the gastric amounts and concentrations of PEG. Conversely, a constant relation between the gastric secretion rate and the emptying rate of the gastric contents leads to a fractional elimination rate of PEG.

The experimentally found semilogarithmically linear curves for the gastric elimination and concentration of PEG are indicative of the existence of a constant relation between the rates of gastric emptying and secretion after a first phase of equilibration. The entering into a semilogarithmically linear decline marks the time, when such a relation is established.

Theoretical support for such a relation is provided by the dual effects on gastric secretion and gastric motility recognized for the enterohormones.

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VI. Influence of the Composition of Food on the Gastric Emptying Pattern

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Quantitative data on the gastric emptying of different meal constituents, hydrochloric acid and gastric contents during the course of emptying an ingested meal are scarce. For studies of the gastric emptying pattern, methods based on the jejunal recovery of gastric and duodenally infused markers are suitable since they do not preclude homogenicity of the gastric contents, do not interfere with normal gastric mixing and propulsion, and do not require repeated tests.

Behavior of the meals in the stomach

Concentrations of test meal constituents in the gastric antrum. After feeding the composite test meals (G, NG, and GP) a separation of the meal constituents occurs in the stomach, evidenced from their concentrations in the successive antral samples (Fig. 1).

The antral concentrations of sugar run parallel to those of the test meal marker (PEG). The mean ratio between the concentrations of sugar and PEG expressed in percent of their concentrations in the meal was 0.99 ± 0.02 in withdrawn antral samples after the G-meal.

The concentrations of fat fell sharply within 30 minutes after intake of all composite meals. This is caused by separation of the fat, which is contained in the upper part of the gastric contents and which, seen from the increasing concentrations towards later periods, reaches the gastric antrum late after ingestion. The separation is less pronounced in the NG-experiments in which, as a result of the early rapid gastric emptying rate, less fat remains in the stomach.

The protein separates from the marker, although to a lesser degree, by the formation of casein coagules. After an early moderate decline the antral concentrations remain fairly constant in consecutive samples. After the protein rich meal (GP) the concentrations are higher, although not proportionally to the increased concentration in the test meal, due to a more rapid dilution by gastric secretion. The gastric concentrations of protein are further influenced by the endogenous contribution. The magnitude of the added protein amount cannot be quantitatively determined after the composite meals. It is probably larger than 0.8 ± 0.1 g, which is the emptied endogenous protein during two hours following ingestion of the protein-free 15 percent glucose solution.

Separation tendency of ordinary meals. To examine whether separation of the different constituents in a meal when mixed with gastric juice is a habit confined to our experimental meals, an in vitro study was performed with two meals from the hospital menu and a home-cooked dinner. After homogenization, PEG was added as a marker and the semi-solid meals were mixed with fresh gastric juice, obtained after stimulation with pentagastrin. The mixture was kept in 37°C for half an hour and the upper and lower parts of the mixture were withdrawn and analyzed for concentrations of PEG, fat, and protein. The results (Table I) suggest that ordinary meals (meal 1 and 3b) like the ones used in this study should not remain homogeneous in the stomach. The results from one of the meals (3a and 3b) suggest that the viscosity of the gastric contents determines the degree of the separation, and this aspect is going to be further examined.

Gastric elimination of test meal marker (PEG)

In the gastric elimination of test meal marker, PEG, (Fig. 2 a–d) several phases could usually be distinguished: an early phase of varying duration and

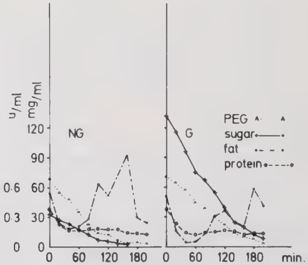

FIG. 1. Average concentrations of fat, protein, carbohydrate (mg/ml) and PEG in successive samples from the gastric antrum after different meals.

TABLE I

In vitro studies with homogenized ordinary meals mixed 1:1 with gastric juice. Samples withdrawn from upper and lower parts of the mixtures were analyzed for concentrations of PEG, fat and protein (mg/ml). Test meal 3 b is meal 3 a diluted 1:1

Meal		PEG mg/l	Fat mg/ml	Protein mg/ml
1	test meal	30.0	62.5	32.3
	upper	13.4	43.8	28.3
	lower	15.0	29.0	22.2
2	test meal	30.0	45.0	16.2
	upper	13.6	12.4	14.6
	lower	15.0	12.0	15.2
3a	test meal	30.0	88.0	33.3
	upper	14.5	45.8	28.3
	lower	13.9	48.0	26.5
3b	test meal	15.0	44.0	15.8
	upper	7.1	43.3	21.2
	lower	8.1	3.3	15.7

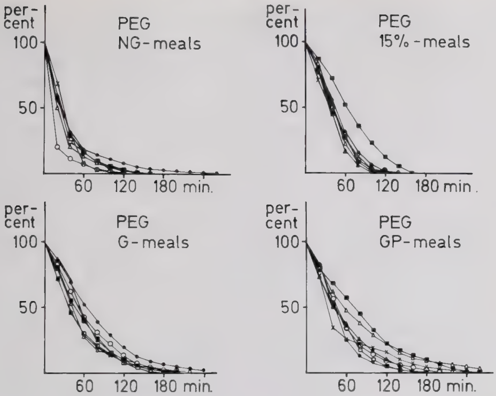

FIG. 2, a, b, c, d. Elimination of test meal marker (PEG) from the stomach after different meals.

character and a second phase recognized by an exponential emptying pattern. A third phase at the end of the experiments was usually discernible.

Significant differences between the meals were present during the early emptying of the marker, which is more rapid after the NG-meal than the glucose-containing meals. The amount emptied during the first 20 minutes following ingestion (47.3 ± 5.8 percent) is higher than after any glucose-containing meal (NG vs G $p < 0.01$, NG vs GP and vs 15% $p < 0.001$).

In four experiments with the NG-meal an initial phase was observed ending within 20 minutes, whereas in three the emptying of PEG described a single exponential course.

After addition of glucose to this meal (G-meal) the early phase was prolonged and characterized by a constant emptying rate of PEG; an average of 22.5 ± 1.5, 27.4 ± 1.5 and 21.8 ± 2.7 percent of the net amount in the test meal were emptied during three initial 20-minute periods.

Increase of the protein content of the G-meal (GP-meal) did not influence the early pattern of emptying of PEG; during three consecutive periods from start an average of 21.4 ± 1.7, 26.0 ± 4.0 and 17.3 ± 3.0 percent respectively left the stomach. Furthermore the linearity of the early phase could be reproduced by the feeding of a 15 percent glucose solution as shown from the mean values for the three initial emptied 20-minute portions 22.5 ± 1.5, 27.4 ± 1.5 and 21.8 ± 2.7 percent.

The exponential phase of gastric elimination of PEG lasted more than two hours after the composite meals. Regressions were calculated between time and the logarithm of the amount of PEG retained in the stomach, the latter expressed in percent of the net amount in the meal. Values from at least five consecutive periods were included, starting at the time of change from the linear to the exponential pattern. The time when the linear phase changes into the exponential phase can be determined in the individual experiments, since at a point where the curve changes from one to the other pattern the rate of emptying is identical according to either curve (1). The total amount of PEG emptied up to the time of change can be derived from

$$100 - P = \text{l.e.r.} \cdot 100/\text{f.e.r.}$$

P	emptying of PEG during the linear phase (percent of the total amount in the meal).
l.e.r.	linear elimination rate (percent emptied per 20 minutes of the total amount in the meal).
f.e.r.	fractional elimination rate (percent emptied per 20 minutes of the amount retained in the stomach).

The fractional elimination rate f.e.r. (Table II) was higher after the NG-meal compared to the twin G-experiment ($p < 0.05$). After doubling of the protein content in the G-meal, the f.e.r. of the marker was not altered. After exclusion of the fat and protein from the composite meals the exponential phase was abbreviated. The f.e.r. after the 15 percent glucose solution were hence higher, than after the G and GP-meal (15% vs G and vs GP $p < 0.001$).

The emptying of PEG proceeded at a faster fractional rate during the last 20 to 60 minutes, thus forming a third phase of the emptying curves. It was, however, too short to be able to be formulated into a mathematical expression.

Comparison of the G and GP-meal versus the NG-meal demonstrates that the presence of a comparably long early phase prolongs the evacuation time of the meal. After the G-meal 97.5 percent PEG had left the stomach after 176 ± 9 min, after the GP-meal 174 ± 19 min, whereas after the NG-meal this amount was delivered in 120 ± 10 min ($p < 0.001$). Due to the abbreviated exponential phase the corresponding figure for the 15 percent glucose solution was shorter than after any composite meal, 110 ± 9 min.

Gastric emptying of carbohydrates, fat and protein. Delivery of calories to the intestine.

The emptying pattern of carbohydrates was identical to that of PEG. After an initial phase of varying duration, carbohydrates left the stomach at constant fractional rates (Fig. 3).

TABLE II

Fractional elimination rate, f.e.r., of test meal marker (PEG, percent emtied per 20 minutes of the amount retained in stomach). Means ± S.E.M. Mean correlation coefficient, r

Test meal	*n*	*r* mean	f.e.r. mean ± S.E.M.
NG	7	0.996	45.9 ± 3.7
G	7	0.997	36.1 ± 1.2
GP	7	0.998	34.4 ± 2.7
15%	7	0.992	61.0 ± 2.5

The emptying patterns of fat and protein are influenced by the intragastric separation, resulting in the emptying of comparably low amounts during the second to fourth 20-minute period. Thereafter, fat and protein left the stomach in constant or slightly increasing amounts throughout the experiments.

A comparison of the T ½ for the different meal constituents (Table III), i.e., the time to empty 50 percent of the net amount in a meal, demonstrates the delayed emptying of fat and protein compared to carbohydrates and shows that the delay is most pronounced for fat.

Significantly higher amounts of fat ($p < 0.01$) and protein ($p < 0.01$) were delivered to the intestine during the first hour following ingestion of the glucose-free composite NG-meal than after feeding the glucose-containing but otherwise identical G-meal (Fig. 3). From the second hour and on, fat and protein left the stomach at equal and approximately constant rates.

Doubling of the protein content of the G-meal did not influence the emptying of fat and carbohydrates, but was followed by the delivery of more protein to the intestine both during the first hour (GP 5.8 ± 0.4 g and G 3.6 ± 0.4 g, $p < 0.005$) and during the ensuing two hours (GP 1.20 ± 0.15 g/20 min and G 0.84 ± 0.05 g/20 min, $p < 0.05$). The latter figures were obtained from regressions between time and the cumulated emptied protein from six consecutive periods.

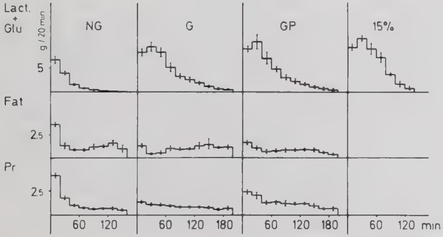

FIG. 3. Gastric emptying of carbohydrates, fat, and protein after the different test meals (mg/20 min).

TABLE III

T ½, i.e., the time (min) to empty 50 percent of the test meal carbohydrate, fat and protein after the different test meals. Mean ± S.E.M.

Test meal	T ½ min		
	Carbohydrate	Fat	Protein
NG	23 ± 2	87 ± 24	32 ± 5
G	48 ± 3	141 ± 10	88 ± 10
GP	47 ± 5	171 ± 26	122 ± 12
15%	43 ± 3		

The transfer of energy to the intestine is demonstrated in Figure 4. Early after ingestion more calories are delivered to the intestine after the NG-meal compared to after the glucose-containing meals (during 0 to 20 minutes NG vs G $p < 0.005$, vs GP $p < 0.025$ vs 15% $p < 0.001$). At 40 minutes from start, the number of calories delivered to the intestine is highest after the meal with the highest energy content (GP) and almost as high after the NG-meal. At 60 minutes the differences between the meals are slight; an average of 127.7 ± 6.8, 141.9 ± 11.2, 158.3 ± 13.0 and 120.5 ± 7.5 kcal had left the stomach after the NG, G, GP-meal, and the 15 percent glucose solution, respectively, during the first hour following ingestion.

The emptying of hydrochloric acid

The delivery of hydrochloric acid after the different meals is demonstrated in Table IV. Regurgitated samples have thereby been included.

Emptying of the gastric contents (test meal plus secretion)

In the emptying pattern of the gastric contents it was usually possible to separate an initial phase, during which differences between the meals and

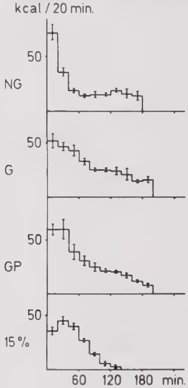

FIG. 4. Rate of delivery of calories to the intestine after different test meals (kcal/20 min).

TABLE IV

Gastric emptying of hydrochloric acid after different test meals (mEq/h).

	Hour		
	1st	2nd	3rd
NG	7.89 ± 1.47	9.75 ± 1.57	9.89 ± 3.73
G	4.66 ± 1.05	6.33 ± 0.77	11.62 ± 2.12
GP	8.38 ± 2.12	11.26 ± 2.66	11.23 ± 2.02
15%	3.80 ± 1.59	11.31 ± 5.75	

differences between individuals given the same meal were pronounced, from a later phase characterized by a low and approximately constant emptying rate (Fig. 5). Following ingestion of the NG-meal, the gastric contents left the stomach at a high early rate of 7.9 ± 1.0 ml/min during the first 20 minutes. The emptying rate declined gradually and was significantly lower ($p < 0.025$) during the third compared to the first 20-minute period. After the first 60 to 80 minutes it remained at a stable level of around 2 ml/min.

Addition of 10 percent glucose to this meal (G-meal) was followed by a significantly lower transfer rate, 3.1 ± 0.4 ml/min ($p < 0.005$) during the initial 20 minutes. This prompt inhibition is gradually diminished during the first hour as demonstrated from the higher emptied volumes during the third, compared to the first 20-minute period ($p < 0.05$). From 60 to 80 minutes after ingestion and on, a low constant emptying rate persisted.

The same early pattern was recognized after feeding the glucose solution, i.e., a prompt inhibition followed by an increasing emptying rate ($p < 0.05$). The volume of the effluent was somewhat higher during the second hour, compared to those recorded after the composite meals, but the difference was not significant due to a considerable individual variation in the handling of the glucose solution.

Doubling of the protein content in the G-meal was not followed by any significant changes in the emptying pattern of the gastric contents.

Discussion

Much effort has gone into describing the characteristics of the gastric elimination of a test meal marker. The elimination curve gives information of the emptying of the meal provided that the ratio between marker and all components of the meal remains constant during the emptying process. However, no reliable conclusions can be drawn from the curve concerning the emptying of the gastric contents (meal plus secretion), which is the more correct resultant of the gastric propulsive activity.

In Hunt's original paper (2) the gastric elimination of marker and of pectin

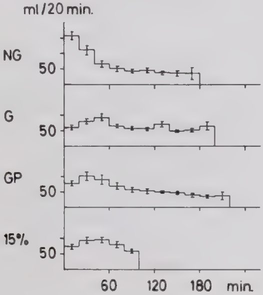

FIG. 5. Emptying of the gastric contents (test meal plus secretion, ml/20 min).

meal was semilogarithmically linear against time. The phase of exponential decline was preceded by an early phase, during which the emptying rate of marker was either higher or lower than could be predicted from the continued course of the curve when plotted as a semilogarithmic function against time. These early deviations were expressed as negative or positive starting indices. The exponential function has been widely accepted as a fundamental pattern of gastric emptying, although in other studies the gastric elimination of a test meal marker has been found to be better adapted to a hyperbolic (3) or square foot (4) or straight linear (5) function against time. The possibility of different interpretations is not surprising in view of the different meals tested and in view of the fact that an elimination curve of a water-soluble marker is the combined result of two processes, namely, the rate of gastric secretion and the rate of emptying of the gastric contents.

The finding of a fractional elimination rate of PEG from the stomach is, as previously discussed (page 43), the expected result of a constant relation between the gastric secretion rate and the rate of delivery of the gastric contents.

The composite meals do not remain homogeneous in the stomach, as demonstrated by the concentrations of meal constituents in consecutive antral samples. The separation delays the emptying of fat and protein which are contained in the upper phase of the gastric contents, compared to the emptying of carbohydrates and PEG which are contained in the watery phase of the gastric contents. Results from in vitro experiments with ordinary meals demonstrate that separation of the constituents in a meal when mixed with gastric juice is of common occurrence.

The use of a water-soluble marker to indicate composite meals has been criticized on behalf of the separation (6). The objection is valid if the quotients between the concentrations of fat or protein and PEG in the test meal are used for calculation of gastric emptying and intestinal absorption. This source of error is avoided, however, in the present study by the use of quotients for gastric concentrations which correspond in time to the concentrations in jejunum. By the sampling from the gastric antrum the concentrations are assumedly representative for the emptied gastric contents.

In a recent paper by our group (7) it was shown that the body weight of a subject determines the rate of delivery of ingested calories to the intestine. Conclusions concerning the emptying patterns of different meals in the present paper are justified by the equal average body weight and distribution of body weights in the investigated groups.

Before proceeding into a discussion of how the gastric emptying is modified by variations in the composition of a test meal, it should be emphasized that the emptying pattern of the marker represents the emptying of carbohydrates but that neither the emptying of the gastric contents nor the loads of food delivered to the intestine are adequately described by an analysis of the emptying pattern of PEG.

The initial and adaptive phase of gastric emptying

The general emptying pattern of PEG and carbohydrates after the glucose-containing meals (*G, GP, 15%*) is similar to that described by Hunt and collaborators (8) after feeding sucrose solutions. They identified an initial phase, followed by a second phase during which the emptying pattern of sucrose (and a water-soluble marker) was fractional. As previously shown by the same group the emptying of disaccharides can be described in terms of their potential monosaccharides and thus comparisons between solutions of di- and monosaccharides can be made. In their study, as after the glucose-containing meals in the present study, the sugar content should be a limiting factor during the early emptying phase. Evidence for this is provided by the significantly lower early emptying rate after the G-meal compared to the NG-meal, the latter being of identical composition except for the exclusion of glucose. Further neither the exclusion of fat and protein from the G-meal as in the 15 percent glucose solution nor the doubling of the protein content as in the GP-meal influenced the early emptying of the carbohydrates and marker. The linear emptying pattern of this early phase is evidenced by the mean values for the first three emptied 20-minute portions of PEG (and carbohydrates).

Summarizing their results from studies with different sugar solutions, Hunt suggested that the amount of sugar in the meal rather than the volume in which it is dissolved, determines the rate of delivery to the intestine, i.e., the transfer of sugar should be regulated according to the load rather than according to the concentration. Their results from plotting the cumulative delivery of sucrose to the intestine supports this reasoning for the range of concentrations used and for the initial phase of emptying. Further support for this view is provided if the data, obtained by Reynell and Spray (9) in rats, are calculated as to the first hourly emptied amount of sugar, this amount being constant over a fairly wide range of concentrations in the given solutions.

Because of the intragastric dilution, the same amount of sugar after the G-meal should be contained in increasing volumes, and according to the discussion above, the emptying rate of the gastric contents should expectedly increase during the first hour. The results agree, in that a higher volume was emptied during the third compared to the first 20-minute period after both the G-meal and 15 percent glucose solution. Knowing the considerable number of interrelated time-dependent variables involved in this complicated feed-back system, any definitive statements on the regulating mechanisms cannot be made at present. In trying to interpret this seemingly load-regulated emptying, one fact is clear, namely, that the net result of the inhibitory influence on the stomach, evoked by the emptied sugar is successively diminished during the first hour. Among factors which might result in this decreasing net inhibition is decreasing velocity at which the emptied meal components travel through the intestine. The mucosal area exposed to the emptied sugar diminishes accordingly due to a more complete absorption at higher intestinal levels when the intestinal chyme is propelled at a decreasing velocity. Another factor is the decreasing concentration of sugar in the gastric effluent. It should be mentioned in this context that the osmolar concentration of the antral contents, for which sugar is mainly responsible, exceeds that of serum at the time when the early and adaptive emptying phase of the glucose containing meals fades into the later state of a constant transfer of the gastric contents and an exponential emptying of the marker.

The inhibitory effect on the gastric emptying evoked by the glucose, has a very rapid onset. The emptying rate is limited already within the first four minutes after the ingestion of the G-meal and is by then significantly lower ($p < 0.005$) than after the NG-meal. The time lapse from the feeding until any signs of an inhibition can be recorded is hence influenced by the composition of the meal.

A comparison of the early emptying pattern of the G and GP-meal reveals that the significantly higher delivery of protein to the intestine after the GP-meal is not accompanied by any decrease of the emptying rate during the first hour.

The early emptying pattern of the carbohydrate poor NG-meal cannot be analyzed from the emptying curves of PEG. The average emptying rate of the gastric contents starts at a significantly higher level compared to that recorded after the glucose-containing meals and declines slowly during the first hour.

No inhibitory influence on the average gastric emptying rate can be discerned within the first five minutes, but, thereafter, an increasing inhibition of the emptying rate can be recognized, reaching its full effect after one hour. Thus if the inhibitory regulation is established more slowly, it results in a continued average emptying rate, which is even lower than after the glucose-containing composite meals, although the somewhat smaller gastric volumes may have contributed to this difference. The inhibitory mechanism should be initiated by the early large emptied portions of the NG-meal, which travel rapidly down the intestine and are exposed to a large mucosal area.

Using data from several studies on gastric emptying, Hunt and Stubbs (10) found that the stomach responds with a decreasing emptying rate of the ingested food, when the caloric density of the meal increases. They used experimental or extrapolated figures for the first half hour emptied caloric portion. Test meals of different compositions and volumes follow the same relation, indicating that the early emptying of any composite meal may be expressed in calories per time. The hyperbolic shape of the curve indicates that the protective mechanisms against the delivery of very high caloric loads to the intestine works well in the low caloric density region. It is less effective when the density of a meal is either very low or exceeds one kcal/ml and any further increase is likely to result in the emptying of more calories per time.

The test meals used in this series are in the range of 0.60 to 1.43 kcal/ml. A comparison of the first hourly delivered caloric load indicates that the emptied load increases with an increasing density of the meal but that differences between meals are small. Comparisons at 40 minutes or less show a lower output after the glucose-containing composite meals compared to the NG-meal, which implies a more prompt inhibition from the intestine to an osmotic than to a caloric load. This time-lag is not surprising, considering that digestion precedes the inhibitory influence exerted by composite food components.

An analysis of the individual experiments shows that the promptness by which the inhibition is initiated influences the time taken for the establishment of a full inhibition. Three experiments with the NG-meal illustrate this tendency (Fig. 6). In Experiment 345 a very low emptying rate after start, which is equal to a prompt inhibition, is followed by a comparably high emptying rate during the ensuing periods and a slow transience into the phase with a constant emptying rate. Experiment 315 demonstrates the opposite situation, namely, that a slow initiation of the inhibition is accompanied by the establishment of a full inhibition already at 40 minutes.

If we accept that the presence of digested food in the duodenum triggers mechanisms by which the continued gastric emptying is progressively inhibited, the logic of the described examples is evident. The early gastric delivery of a larger caloric load (as in Experiment 315) should elicit a larger inhibitory response and consequently an earlier establishment of a full inhibition.

The continued pattern of gastric emptying

During the continued emptying process fat and protein are delivered to the intestine at approximately constant rates after all composite meals. The output rates of pancreatic enzymes are constant (11) and the intestinal transit slower (12). Since the absorption is almost completed within the proximal 70

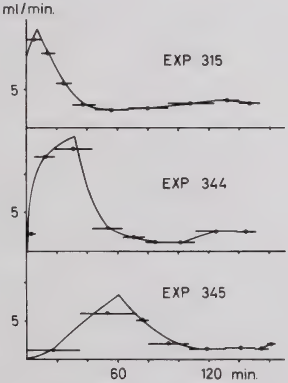

FIG. 6. Emptying of the gastric contents (test meal plus secretion) in three experiments with the NG-meal (ml/min).

cm of the intestine (13), only minor amounts of the meal components travel far down the intestine. The stimulus to the release of factors regulating the gastric emptying should therefore be maintained at a fairly even level, resulting in a constant transfer of the gastric contents. The experimentally found constant rates at which the gastric contents are delivered to the intestine after the composite meals support this interpretation of events. This concept of an established "steady state" is useful in the description of the general course of events in the continued gastrointestinal dealing with the meals, and allows comparisons between the different meals. From the results obtained in the individual experiments it is realized that the "steady state" is an approximation and that continuous small adaptive changes occur in all variables.

We cannot establish from this study by which of the meal components the continued gastric emptying is limited. Since the continued phase was abbreviated in the experiments with 15 percent glucose solution, it seems probable that the fat or protein content of the composite meals prolongs the gastric emptying. Fat is the suspected component since it has long been known that fat is a potent inhibitor of the gastric emptying rate as well as of the gastric secretion, and since the studies by Knox and Mallinson (14) it has been recognized that fat exerts its inhibitory influence after being digested. This should be in line with the recent suggestion by Moberg and Carlberger (15) that the emptying of their composite meal was regulated by the action of fat within the small intestine.

If the protein content of the meals was responsible, we should expect that an increase of the protein content should decrease the gastric emptying rate. This, however, was not the case in the series of experiments in which the protein content of the G-meal was doubled. Despite a higher gastric output of protein both during the initial and continued phase, the emptying of the gastric contents proceeded at the same rate as after the G-meal. If an increased inhibition is evoked by the higher protein output, it is evidently counterbalanced by an equal increase of factors facilitating the gastric emptying.

An alternative explanation includes the concept of a maximal inhibition which is congruent with the hyperbolic relation between the caloric density of a meal and its rate of transfer to the intestine stated by Hunt. If such a maximal inhibition is set up by the G-meal, any increase in its nutritional content could not further influence the gastric emptying rate, but should lead to changes in the intestinal dealing with the meal. The shorter transit times and the higher pancreatic and intestinal secretion recorded after the GP-meal compared to the G-meal support this reasoning.

In the series with the G and NG-meal the individual maximum absorption rates during transit of the investigated segment were reached early after ingestion whereas considerably lower absorption rates were recorded during the second phase of gastric emptying (13). If the absorption rate per se limited the gastric emptying rate, we should expect to find the same absorption rate throughout the experiments, and consequently a considerably higher emptying rate. The results therefore imply that the absorption rate is determined by the gastric emptying rate rather than the reverse.

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VII. Intestinal Flow Rates and Mean Transit Times and Their Relation to Gastric Emptying

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It is probable that the intestine is sensitive to cephalic vagal stimulation evoked by eating and by hormones released by the presence of food in the stomach. Nevertheless, it seems more reasonable to attribute the main motor, absorptive, and secretory response from the intestine to the delivery of food from the stomach. Therefore, the intestinal flow rates and mean transit times are discussed in relation to the gastric emptying of the four meals studied.

General pattern of intestinal flows and transit times. The experiments were continued until the test meal marker (PEG) had disappeared from the jejunal chyme. At this time usually only small volumes could be siphoned from the sampling level. After the G and GP-meal the jejunal flow could usually be followed for more than four hours, whereas the experiments with the NG-meal and glucose solution lasted shorter. The jejunal concentrations of total indicator varied after a slow rise during the first one to two hours (Fig. 1). Due to individual variations in gastric emptying rates and transit times the timing of the jejunal peak flow rate differed between the subjects, the maximum usually being reached within 80 minutes after meal ingestion. The means of the individual peak flow rates did not differ between the NG and G-meal but were higher after the GP-meal (GP vs G, $p < 0.05$) and after the glucose solution (15% vs G, $p < 0.02$). Although fairly even flow rates were obtained in some experiments during the subsequent hours, variations were more frequently observed. There was no constant relationship between the total indicator concentration and the flow rate during the course of the experiments.

Like the concentration of total indicator, the mean transit time followed an undulatory course, usually in the same direction as the changing concentrations but not coincident in time (Fig. 1).

Mean transit times. The first arrived portions of the duodenal indicator and of test meal marker usually represent the fastest particles of the infused or emptied amounts and have accordingly transited the segment at a high speed. During the ensuing periods the transit times slowly increase, i.e., the emptied gastric contents are propagated more slowly through the investigated segment.

The undulatory changes of the mean transit time are evidently lost in the mean values (Table I), yet the latter form a basis for a comparison between the meals. It is thereby seen that the average transit times are very similar after the NG and G-meal. Compared to the G-meal, shorter transit times were recorded through the entire course of the GP-meal and the glucose solution.

Jejunal fluid flows. The higest average flow rates (Table II) ranged from 5 ml/min during the third 20-minute period after the G-meal to 10 ml/min during the second after the GP-meal. From the second to third hour and on,

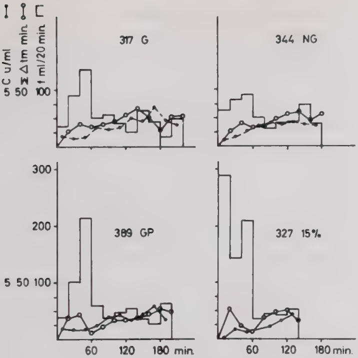

FIG. 1. General pattern of intestinal variables in four experiments, one with each test meal. Concentration of total indicator, *C* units/ml, postprandial flow rate at the investigated jejunal level, f ml/20 min. The mean times to travel the segment for the amounts of duodenal indicator which have reached the jejunal sampling site, *tm* min, are constructed by the continuous addition of the changes of the mean transit time during consecutive 20-min periods, Σ Δ *tm* min.

TABLE I

Concentrations of the duodenal indicator (C units/ml) in jejunal samples and mean times for consecutive portions of indicator to transit 70 cm of the proximal intestine, (tm, min). Means ± S.E.M. from seven experiments with each test meal.

per nr	NG		G		GP		15%	
	tm min	*C* u/ml	*tm* min	*C* u/ml	*tm* min	*C* u/ml	*tm* min	*C* u/ml
1	5.9 ± 0.8	0.70 ± 0.23	7.1 ± 0.9	0.69 ± 0.17	6.8 ± 1.3	0.9 ± 0.2	7.0 ± 1.0	0.5 ± 0.1
2	15.3 ± 2.2	0.69 ± 0.05	18.1 ± 2.5	1.01 ± 0.26	15.7 ± 1.3	0.8 ± 0.2	13.6 ± 3.0	0.7 ± 0.1
3	19.8 ± 2.9	1.14 ± 0.10	20.3 ± 4.0	1.05 ± 0.22	19.0 ± 6.3	1.2 ± 0.4	13.2 ± 2.4	0.8 ± 0.2
4	23.9 ± 4.2	1.73 ± 0.16	22.2 ± 4.2	1.56 ± 0.28	21.1 ± 8.0	1.4 ± 0.3	13.0 ± 2.2	1.1 ± 0.1
5	25.6 ± 4.8	2.29 ± 0.15	26.3 ± 3.4	2.03 ± 0.38	22.3 ± 8.1	1.6 ± 0.2	18.6 ± 3.8	1.2 ± 0.1
6	25.6 ± 4.6	2.22 ± 0.15	30.6 ± 3.8	2.25 ± 0.29	22.4 ± 8.3	1.8 ± 0.3	23.4 ± 5.3	2.5 ± 0.3
7	26.8 ± 3.9	2.07 ± 0.06	31.3 ± 4.3	2.56 ± 0.19	23.5 ± 8.7	1.9 ± 0.3	25.9 ± 6.7	3.5 ± 0.8
8	29.1 ± 3.8	2.10 ± 0.18	29.6 ± 5.4	1.50 ± 0.29	24.7 ± 8.2	2.2 ± 0.5	29.0 ± 8.8	4.3 ± 1.1
9	37.4 ± 4.0	2.62 ± 0.40	27.8 ± 5.6	2.60 ± 0.30	25.1 ± 7.4	2.3 ± 0.6		
10	44.3 ± 4.0	3.23 ± 0.47	32.6 ± 5.8	2.34 ± 0.13	26.5 ± 7.5	2.5 ± 0.5		
11			36.1 ± 6.1	2.26 ± 0.18	32.6 ± 10.9	2.5 ± 0.5		
12			34.5 ± 7.2	2.52 ± 0.23				
13			24.1 ± 1.1	3.11 ± 0.32				

average flow rates of about 2 ml/min persisted throughout the experiments with only minor differences between the different meals.

Equal flow rates were recorded after the NG and G-meal. Doubling the protein content in the G-meal was followed by higher flow rates at the investigated jejunal level, the first hourly fluid volume after the GP-meal amounting to 403 ± 77 ml compared to 225 ± 29 ml after the G-meal ($p < 0.05$). The continued flow rate was also higher after the GP-meal, and the test

TABLE II

Flow rates at the jejunal sampling site (f, *ml/20 min*) *and water loads from the stomach corresponding to each jejunal period* (L, *ml/20 − Δ tm min*). *Means ± S.E.M. from seven experiments with each test meal.*

per nr	NG		G		GP		15%	
	f ml	*L* ml	*f* ml	*L* ml	*f* ml	*L* ml	*f* ml	*L* ml
1	63 ± 17	45 ± 17	34 ± 8	10 ± 3	90 ± 43	14 ± 9	130 ± 39	27 ± 9
2	119 ± 20	120 ± 10	90 ± 19	49 ± 13	197 ± 44	100 ± 27	128 ± 59	67 ± 16
3	74 ± 15	100 ± 21	102 ± 8	87 ± 11	116 ± 32	80 ± 23	161 ± 27	123 ± 25
4	48 ± 9	53 ± 3	72 ± 20	72 ± 10	82 ± 23	72 ± 20	91 ± 24	65 ± 12
5	46 ± 8	49 ± 12	54 ± 16	57 ± 11	71 ± 15	59 ± 8	63 ± 16	66 ± 18
6	44 ± 3	41 ± 5	39 ± 5	50 ± 10	63 ± 11	56 ± 5	37 ± 5	45 ± 11
7	46 ± 7	40 ± 8	46 ± 8	63 ± 13	62 ± 16	51 ± 6	32 ± 7	50 ± 17
8	42 ± 7	42 ± 8	45 ± 5	58 ± 10	56 ± 9	46 ± 5		
9	28 ± 6	32 ± 8	34 ± 3	51 ± 5	57 ± 12	45 ± 9		
10	28 ± 5		35 ± 7	47 ± 10	47 ± 10	35 ± 6		
11			41 ± 8	58 ± 13	43 ± 10			
12			48 ± 10	48 ± 7				
13			27 ± 4					

meal marker (97.5 percent) passed in an average volume 851 ± 126 ml, which significantly ($p < 0.025$) exceeds the corresponding volume of 503 ± 49 ml for the G-meal. Early high flow rates were obtained after feeding the glucose solution amounting to 418 ± 57 ml during the first hour (15% vs G $p < 0.01$).

Passage of gastric and intestinal markers at the sampling level. The fluid flow at a jejunal level is composed of nonabsorbed test meal and secretions from the stomach, pancreas, and intestine. Comparison of the passage of test meal marker and the duodenally infused indicators at the investigated level makes a separation possible between the gastric and intestinal influence on the management of a meal (Fig. 2).

The bulk of PEG passed earlier at the jejunal sampling level after the NG-meal compared to the G-meal; the figures for the first hour were 149 ± 6 and 83 ± 10 units, respectively ($p < 0.001$). The difference can altogether be attributed to the different gastric emptying rate, since an equal amount of the duodenally infused indicator passed during the same time 184 ± 21 units after the NG-meal and 198 ± 24 units after the G-meal.

After feeding the glucose solution, more PEG (15% vs G $p < 0.05$) and more of the duodenal indicator (120 ± 11 units) passed the sampling level within one hour after feeding. A comparison of the GP and G-meal as to the cumulative passage of PEG and duodenal indicator reveals higher figures after the protein-rich meal in all consecutive periods during two hours after meal ingestion. The differences should result from different transport rates through the segment, since PEG leaves the stomach at equal rates throughout the G and GP-experiments and during the first hour after the G-meal and glucose solution.

From Figure 2 it is further demonstrated that the passage of 200 units PEG at the investigated jejunal level is a slower process after the G and GP-meal compared to after the NG-meal and glucose solution.

The amount of PEG in the investigated 70 cm intestinal segment is equal to the cumulative gastric emptying of PEG subtracted by the cumulative passage at the jejunal sampling level (Fig. 3). During the first two 20-minute periods the segment contained more PEG after the NG-meal than after the G-meal. At 80 minutes and during all subsequent periods, more PEG was present in the segment after the G-meal ($p < 0.005$–0.05). Compared to the G-meal, less PEG was retained in the segment throughout the entire experiments with the glucose solution and during the first two hours after feeding the GP-meal, evidently the consequence of a more rapid transit.

Intestinal net secretion. The amount of PEG that passed the jejunal sampling site during the 20-minute period left the stomach tm_n minutes earlier during $20 - \Delta tm_n$ minutes. Since the antral concentrations of PEG are known, we know the volume in which the emptied PEG was contained, i.e., the water load from the stomach, corresponding to the jejunal period n. The difference between the flow volume at the jejunal sampling level and the

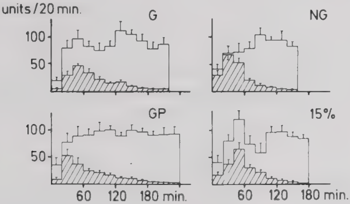

FIG. 2. Means ± S.E.M. for the distribution of test meal marker, PEG (hatched), in the intraduodenally infused indicator at the sampling site in jejunum, units/20 min. The concentrations of PEG are, like those of the duodenal indicators, converted into a relative unit/ml. The net amount of PEG in the test meal has the magnitude 200 units and the amount of indicator infused during 20 minutes 100 units.

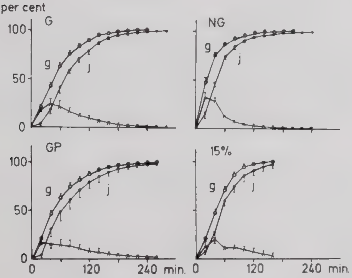

FIG. 3. Subtracting the figure for the cumulative passage of PEG in jejunum (j) from the figure for the cumulative emptying of PEG from the stomach (g) gives the amount of PEG present in the investigated segment at time intervals (percent of the net amount of PEG in the test meal).

corresponding emptied volume (Table II) represents the net water secretion or absorption during transit.

A variable net water exchange was recorded after the experiments with the NG-meals, the tendency being a net dilution of the first 20-minute jejunal flow and a net absorption thereafter, resulting in an average net absorption of 10 ± 22 ml from the gastric load, corresponding to the first hourly jejunal flow. In contrast significant water volumes had been added to the first and second 20-minute flow volumes after the G-meal (26 ± 6 ml and 41 ± 10 ml respectively), and the average net secretion to the first hourly volume was 82 ± 22 ml (G vs NG $p < 0.025$). An even higher net secretion was recorded during the first hour after both the GP meal (209 ± 51 ml, GP vs G $p < 0.05$) and the glucose solution (201 ± 51 ml).

Except for the GP-meal, after which a small average net secretion persisted during the entire experiment, the jejunal fluid flows were about equal to the emptied volumes after the first hour, indicating a balance between the secretion and absorption of water in the upper intestine.

Intestinal mixing. The mixing index varied considerably both within and between experiments, making definite conclusions regarding specific patterns hazardous (Table III). As expected, the degree of mixing usually increased from the second to the third and fourth indicator. It should be noted that despite the shorter transit times after the GP-meal, the mixing indices were higher for all three indicators compared to those after the G-meal.

Discussion

In discussing variations of the flow rates and mean transit times, it must be emphasized that the jejunal sampling site, as in all intubation techniques, is functionally rather than anatomically defined. Telescoping of the tube may occur during the experiments, although it should be moderate with tubes of the small diameter used (1). The rather constant or even decreasing mean transit times towards the end of the experiments indicate that this complication is of limited importance.

In the individual experiments jejunal peak flow rates occurred within 80 minutes after ingestion of either meal, usually tm minutes after delivery from the stomach of the highest caloric loads. During the continued experiments, the flow rate varied. The undulatory course of the variations indicates that they are real and not caused by nonrepresentative sampling. Also the variable

TABLE III

Mixing indices for the intraduodenally infused indicators after different meals. Mean ± S.E.M.

Test meals	NG	G	GP	15%
Indicator nr				
II	1.69 ± 0.13	1.61 ± 0.13	2.17 ± 0.46	1.80 ± 0.20
III	2.06 ± 0.31	2.28 ± 0.27	2.48 ± 0.57	2.65 ± 0.57
IV	2.32 ± 0.29	2.15 ± 0.21	2.69 ± 1.05	

flow rate corresponds to observed variations in the sampling volumes, and agrees with the common observation of intermittent flows through intestinal stomas. Further support for a variable flow rate is the finding of an uneven distribution of $Cr^{51}O_4Na_2$ in the rat intestine by Derblom, Johansson, and Nylander (2).

The early high fluid volumes recorded after all glucose containing meals are due to a comparatively higher degree of dilution in the proximal intestine, assumedly the result of a stimulatory action of glucose or osmoles on the duodenal secretion.

The dilution of a hypertonic meal during passage of the upper intestine was previously demonstrated by Fordtran and Ingelfinger (3) who estimated the average jejunal fluid flows from the dilution of a test meal marker. Their results inspired a comparison with G-meals in the present series between the average flow rate values in each experiment, calculated: a) as a mean of the computed fluid flows, and b) from dividing the emptied PEG by the sum of the concentrations of PEG in jejunal samples. The latter differed by an average of +7 ml per 20 minutes (range −7 ml to +14 ml/20 minutes, *n*:7, G-meal), which was not significant. Calculation of an average flow rate from the sum of concentrations in jejunal samples was thus a valid estimator of the average fluid flow at the sampling level due to the fact that an underestimation of the flow volumes during the first part of an experiment is counterbalanced by an overestimation in later periods. This is analogous to the finding when mean values for the absorption obtained with the present method are compared to those obtained with semiquantitative techniques.

The mean transit time, tm, is regarded as a continuous function against time of the added changes of tm per 20 minutes. Within the limit for calculation method, tm is valid from the start of the experiments for the duodenal infused indicator and, by the inclusion of the concentrations of PEG in the flow calculations, assumedly also for PEG and test meal components. From the start tm is also valid for the duodenal secretions added after intake of the meal. However, not until the whole intestinal residual volume has left the examined segment is the mean transit time representative for every fluid particle at the investigated level, and only then a valid expression for the average speed with which the intestinal contents are propelled.

The initially arrived portions of the meals traveled through the investigated segment at a high velocity. During the ensuing periods the transit time slowly increases, and is by then in fact representing a slowing of the propulsion of the intestinal contents. Any conclusions drawn concerning this slowing are premature, yet some differences between the meals deserve mentioning. After the NG-meal the slowing was somewhat less prompt than after the G-meal, which results from the very high initial gastric emptying rate of the former meal. The slowing was more prompt after the G-meal compared to the glucose solution, indicating that the emptied fat and protein might have a slowing effect on the propagation of the intestinal contents. On the other hand an increase of the protein content in the G-meal did not further increase the transit times. Rather the opposite was seen in that the mean transit times were shorter, and as late as at the end of the second hour transit times shorter than 10 minutes were observed in three GP-experiments. Among possible interpretations it seems most probable that the slowing effect of the fat in the GP-meal is overcome by an accelerating effect of the emptied large amounts of protein either through a direct action or through distension of the proximal intestine by secretions stimulated by the digested protein.

During the continued transfer of the meals the transit times decreased and increased smoothly usually over several periods. A similar infrequent acceleration of the propagation speed, as indicated by the downward slopes of the undulations was observed by Barreiro, McKenna, and Beck (4) when a propulsive wave engages an entire intestinal segment. In their study the intraluminal pressure changes along an intestinal segment were recorded during simultaneous perfusion of the segment.

Comparison of the NG and G-meal. The earlier passage of PEG at the investigated intestinal level after the NG-meal corresponded to the higher early gastric emptying rate of this meal compared to the G-meal. Despite this, only minor differences were recorded in the first hourly fluid volumes at the jejunal sampling site because of a larger dilution of the hypertonic G-meal in the proximal intestine, which was still measurable as a net secretion at the jejunal sampling level. This dilution of the G-meal in the intestine compensates for the different emptying rates of the two meals, and, by equalizing the volumes in the proximal intestine, accounts for the similar passage rate of the duodenal indicator in jejunum at one hour from start and, consequently, for the similar transit times. The findings demonstrate that no conclusions can be drawn about the effects of differences in gastric emptying rates on intestinal transit if intestinal secretion and absorption are not considered.

Comparison of the protein-rich GP-meal and the G-meal. The only difference in the gastric emptying of the two meals is the delivery of higher amounts of protein to the intestine after the protein-rich GP-meal, particularly during the first hour after ingestion.

The higher gastric emptying rate of protein was followed by a larger net secretion and consequently higher flow rates at the jejunal level. The larger net secretion could be due to a higher secretion from the pancreas and duodenum, to a decreased absorption or to both. It is probable that the secretion is higher, since the output of pancreatic enzymes was higher after the GP-meal and protein is a known liberator of both cholecystokinin and secretin. It is also probable that the absorption of the secreted water is less complete because of the shorter transit times after the GP-meal.

Comparison of the glucose solution and the G-meal. As previously reported carbohydrates like PEG and water left the stomach at equal rates during the first hour after the two meals, and the osmotic loads to the intestine were therefore of comparable magnitudes. The stimulus to intestinal secretion should thus be rather equal between the meals. The first hourly jejunal fluid volumes (and the amounts of PEG), however, were significantly higher after the glucose solution compared to after the G-meal, and more sugar passed the jejunal sampling site. A less complete absorption of the intestinal secretions and sugar (5) during the shorter transit times after the glucose solution could explain the higher jejunal fluid volumes. As demonstrated in Chapter IX Figure 4, the segmental absorption of sugar is less efficient when the transit is rapid and is very limited at transit times lower than 15 minutes.

Towards the end of the experiments with the 15 percent glucose solution the *tm:s* and the jejunal flow rates approached those of the G-meal. Considerable differences as to the gastric caloric loads exist at this time; so was the delivery of calories during 80–140 minutes after intake of the glucose solution less than one-third of that recorded after the G-meal, and the absorption of glucose completed at intestinal levels proximal to the jejunal sampling site.

Fate of the meal in lower gastrointestinal tract. Although the experimental data give quantitative information only concerning the events in the upper part of the intestine, they can be used to extrapolate the transit of the duodenal indicator and test meal marker (PEG) through the entire intestine and the distribution of PEG in consecutive intestinal segments. Such estimates can be made from the mean values to create a general picture of the continued fate of the meal. Evidently this picture cannot provide conclusive material concerning the individual response to food, since the continuous adaptive variations are lost in the calculations.

The intestine was divided into compartments, each representing one transit time. The calculations were based on the assumption of a linear velocity of the indicator particles, i.e., if the particles arrive at a distance of 70 cm from their entrance site after *tm* minutes, they will arrive at 140, 210, and 350 cm after 2, 3, and 5 *tm*, respectively. The amount of PEG that was emptied during $20 - \Delta\ tm_n$ minutes passed at 70 cm during 20 minutes and will consequently pass at 140 cm during $20 + 7\ \Delta\ tm_n$ minutes, and at 350 cm during $20 + 4\ \Delta\ tm$ minutes. This assumption implies a proportionality between the changes of the fluid flows and intestinal volumes along the intestine and is supported by the proportional decrease of volumes and flow rates at flow rates less than 7 ml/min found by Dillard et al (6). Support is further provided by the analogy between the transit time through the proximal intestine (from our data) and the length of the intestine (from the data of Borgström et al as to the efficiency of sugar absorption Chapter IX, Figure 7).

The high velocity with which the first emptied portions travel through the proximal intestine results in a rapid spreading far down the intestine. A considerable difference exists between the fastest and slowest particles in a portion because of the great changes in the mean transit time during early periods. Later, when *tm* is small, the passage rates of PEG are more similar at higher and lower intestinal levels.

The distribution of the test meal marker in consecutive 70-cm segments of the intestine (Fig. 4) was similar to that found in rat intestine after intragastric administration of radioisotope by Nylander, Derblom, and Johansson (2) and by Poulakos and Kent (7). This applied especially to the

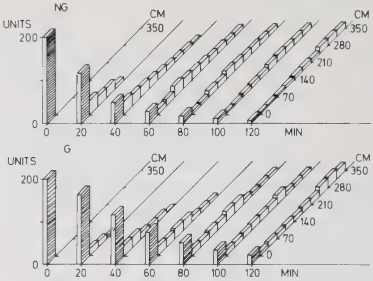

Fig. 4. The distribution of test meal marker (PEG) in consecutive intestinal segments at different times after the NG and G-meals. Extrapolation made according to text.

distribution of PEG after the NG-meal and may be ascribed to the similar exponential gastric emptying pattern of marker. The correctness of the assumption was tested against the results by Mattsson, Perman, and Lagerlöf (8) who examined fluoroscopically the intestinal transit of the G-meal. The calculated time both for appearance and disappearance of PEG in the distal ileum agreed with their results. However, it should be noted that their meal contained a barium sulphate suspension which, by giving more bulk for the distal intestine to propel, might have influenced its transit and thus invalidates the comparison.

Another possible extrapolation is based on the assumption of a decreasing velocity of the indicator particles along the intestine. It could not be tested since it precludes knowledge of the intestinal volumes and flows at different levels. It would result in a growing accumulation of the duodenal indicator in distal intestinal segments and thus confirm the finding by Grossman of a higher amount of pancreatic enzymes in distal compared to proximal segments of the intestine in fed rats (9). It must be emphasized that this finding is not inconsistent with a linear velocity. A somewhat less pronounced accumulation of enzymes will occur in distal parts of the intestine at the beginning and at the end during the passage of a meal.

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VIII. Output of Pancreatic Enzymes and of Bilirubin and Their Relation to Gastric Emptying of Different Test Meals

KERSTIN EKELUND AND CATJA JOHANSSON

Jejunal concentrations of pancreatic enzymes and of bilirubin. No significant differences were found between the jejunal concentrations of the different enzymes. Siphoned volumes of jejunal contents were sometimes too small to permit all chemical analyses. Among the pancreatic enzymes lipase was most often determined and we therefore selected lipase to describe the pattern of enzyme output. Except during the first 40 minutes, the concentrations of lipase are highest after the GP-meal, lower after the G-meal, and lowest after the 15 percent glucose solution (Fig. 1). The concentrations of bilirubin are initially high after the GP and the G-meal and thereafter decrease, and later on the concentration-time curves follow an undulatory, slightly increasing course (Fig. 2).

Output of pancreatic enzymes and of bilirubin. The highest output rate of lipase was found after the GP-meal (Fig. 3a). It was lower after the G-meal and lowest after the 15 percent glucose solution. The output rate of bilirubin was also higher after the GP-meal compared to the G-meal and the 15 percent glucose solution (Fig. 3b). The total output of lipase (i.e., the sum of output from zero time up to the time when 97.5 percent of the test meal marker is emptied) was larger after the GP-meal than after the G-meal ($p < 0.05$) and the 15 percent glucose solution ($p < 0.01$).

No significant differences were found regarding the output of bilirubin and pancreatic enzymes during the first hour. During the second hour, however, the output of lipase and bilirubin was lower after the 15 percent glucose solution compared to the two composite meals (lipase: G and GP vs 15% $p < 0.01$, $p < 0.01$, bilirubin: G and GP vs 15% $p < 0.01$, $p < 0.01$; Table I).

For the investigated meals the output of lipase during the first hour was larger compared to that of the second hour (G $p < 0.05$, GP $p < 0.05$, 15% $p < 0.01$). The same was found for bilirubin after the G-meal ($p < 0.02$) and the 15% glucose solution ($p < 0.02$). In Figure 4 a and b is seen the output of lipase and bilirubin from the 5 subjects investigated with the G and the NG-meal on two different occasions. The output rates of bilirubin and of lipase are higher after the NG-meal compared to the G-meal, the differences in output did not reach a significant level.

Discussion

The effect of protein on pancreatic and biliary response was evaluated after ingestion of a high protein meal (GP) and a low protein meal (G). The emptying rate of fat and sugar was approximately equal after the two meals

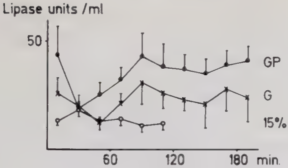

FIG. 1. Jejunal concentrations of lipase (L.U./ml, Mean ± S.E.M.).

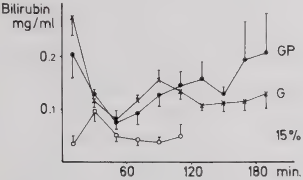

FIG. 2. Jejunal concentration of bilirubin (mg/ml, Mean ± S.E.M.).

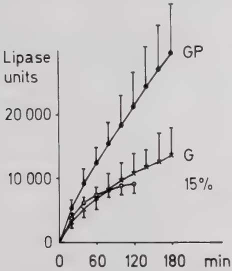

FIG. 3a. Cumulative output of lipase after the G, GP-meal and the 15 percent glucose solution (L.U. Mean ± S.E.M.).

but that of protein faster after the GP-meal (1). The delivery of more protein per time unit was followed by a higher output rate of enzymes and of bilirubin. During the first hour the gastric emptying of hydrochloric acid (being 4.1 ± 1.1 mEq after the G and 9.3 ± 2.3 mEq after the GP-meal) may have influenced the enzyme and bilirubin output (2) this should not be the case during the second and third hour since equal amounts of acid were delivered to the intestine (being 9.5 ± 1.6, 13.9 ± 1.6 mEq after the G-meal and 12.3 ± 2.3, 12.5 ± 1.0 mEq after the GP-meal. Note: G n:8, GP n:6).

Another conclusion to be drawn from this series concerns the influence of glucose on pancreatic secretion of enzymes and the biliary output. The suggestion by Hong, Chin, and Hur (3) that the pancreatic secretion is

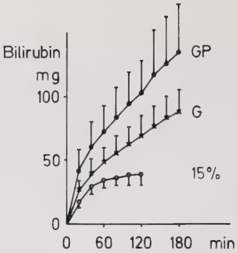

FIG. 3b. Cumulative output of bilirubin after the G, GP-meal and 15 percent glucose solution (mg, Mean ± S.E.M.).

TABLE I

Output of pancreatic enzymes and of bilirubin per hour after three different test meals (mean ± S.E.M.).

		Average enzyme output per hour		
		1st	2nd	3rd
$G_{n \cdot 8}$	Amylase (AU)	1121 ± 182	635 ± 115	427 ± 96
	Lipase (LU)	6926 ± 1942	3788 ± 1080	2594 ± 923
	Trypsin (mg)	168 ± 44	142 ± 34	109 ± 24
	Bilirubin (mg)	48 ± 12	21 ± 4	21 ± 5
GP	Amylase	1670 ± 394	1006 ± 213	854 ± 210
	Lipase	12283 ± 3114	8828 ± 2515	8320 ± 2238
	Trypsin	291 ± 62	202 ± 17	196 ± 26
	Bilirubin	72 ± 22	31 ± 7	29 ± 9
15%	Amylase	834 ± 179	272 ± 111	
	Lipase	7364 ± 975	2019 ± 529	
	Bilirubin	34 ± 6	6 ± 2	
$G_{n \cdot 5}$	Amylase	1106 ± 282	696 ± 174	555 ± 103
	Lipase	5979 ± 2767	3409 ± 1704	3300 ± 1405
	Trypsin	153 ± 61	138 ± 53	141 ± 19
	Bilirubin	32 ± 8	16 ± 4	23 ± 6
NG	Amylase	1252 ± 349	646 ± 150	
	Lipase	8203 ± 2129	5462 ± 1602	
	Trypsin	196 ± 42	125 ± 24	
	Bilirubin	47 ± 7	26 ± 10	

depressed after ingestion of a glucose solution cannot be supported by our data. The finding of a considerable early output of bilirubin and of pancreatic enzymes after ingestion of a 15 percent glucose solution is in line with the results of Young, Lazarus, and Chisholm (4), who found increasing levels of pancreozymin and of secretin within 20 minutes after peroral loads of glucose. Whether the early high output should be ascribed to a stimulatory effect exerted by glucose per se and/or if it results from volume distention of the stomach and/or through the action of hypertonic duodenal contents can at the present not be concluded. From the second hour and on, the pancreatic response to the glucose solution was small, which is consistent with the suggestion by Grossman and Wang (5) that isotonic glucose solutions only weakly, if at all, stimulate pancreatic secretion of enzymes.

It should be noted that the three meals discussed so far contain the same amount of potential monosaccharides. The influence of sugar was further investigated because 5 of the 8 subjects investigated with the G-meal were on another occasion investigated with the same type of meal without glucose, i.e., the NG-meal. The ingestion of the NG-meal was followed by a delivery of more fat and protein to the intestine during the first hour, resulting in a higher pancreatic secretion rate of enzymes and in a higher output rate of bilirubin during the course of the experiments. The total amount of enzymes and of bilirubin did not differ after ingestion either of the G or NG-meal because the G-experiments lasted for a longer time than the NG-experiments. The effect of glucose per se on the pancreatic and biliary response seems to be negligible when contained in a composite meal.

After all meals the initial pancreatic and biliary response was higher than during the continued experiments. This is due to a washout effect and to the early rapid delivery of food constituents to the intestine and/or volume distention of the stomach and the intestine. The quantitative differences in pancreatic secretion are influenced by quantity and quality of food delivered to the intestine. The average pancreatic output of amylase after the high protein meal (GP-meal) is equal to that obtained by Schütz, Andersson, and

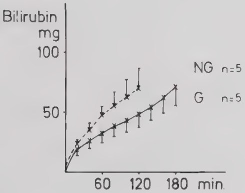

FIG. 4a. Cumulative output of lipase after the G and the NG-meal (L.U. Mean ± S.E.M.).

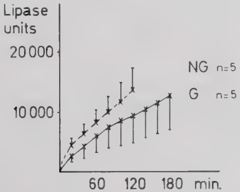

FIG. 4b. Cumulative output of bilirubin after G and the NG-meal (mg, Mean ± S.E.M.).

Lagerlöf (6) after maximal stimulation with pancreozymin and secretin. The first hourly output of enzymes after both the low protein meal (G-meal) and the 15 percent glucose solution was about 60 percent of the maximal response.

During the first two hours after maximal stimulation with secretin and pancreozymin, Schütz (unpublished data) recorded a bilirubin output of 71 ± 7 mg (n:7) which is lower than observed after the protein-rich meal in this series. The total daily output of bilirubin has been estimated to be about 220 mg (7). It is notable that moderate caloric loads like the GP-meal (429 kcal) and the G-meal (387 kcal) given to fasting subjects in the morning resulted in an output of bilirubin no less than 55 and 41 percent of the calculated daily output.

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IX. Absorption of Sugar, Fat, and Protein in the Upper Intestine in Relation to Loads, Transit Times, and the Endogenous Dilution

CATJA JOHANSSON

The present concept of the intestinal absorption in man after meal intake is based on the classic work by Borgström, Dahlqvist, Lundh, and Sjövall (1). After feeding a meal of essentially the same composition as the G-meal in the present study they observed that the absorption was almost completed in jejunum, the site of absorption being more proximal for fats than carbohydrates and more proximal for carbohydrates than proteins. Their results have been confirmed by others (2) using the same semiquantitative inhibition technique. These techniques can be used to get an average figure of the absorption but do not provide information of the absorptive pattern during the course of digestion and absorption of a meal, neither its relation to gastric emptying or to the intestinal transit time, the latter being a neglected factor in all hitherto used techniques for studies of absorption. (For explanations of definitions and symbols, see Figure 2 legend or Chapter III.)

Jejunal contents. The pH of the jejunal contents was around 6. No decrease was observed in later periods, when the acid load from the stomach was largest.

After the carbohydrate-poor NG-meal the jejunal concentrations of PEG (Fig. 1) reached an early peak within the first hour and declined slowly thereafter. After the glucose-containing composite meals the peak concentrations were reached later and were lower ($p < 0.005$). The difference is partly due to the lower delivery rate of PEG from the stomach and partly to the larger dilution of these meals when they pass the jejunal sampling level. After 1 to 1½ hours the jejunal concentrations of PEG are only slightly lower than or equal the corresponding gastric concentrations *tm* minutes earlier, indicating that the water volume added during transit of the proximal intestine only slightly exceeds or equals the absorbed.

The decline of the jejunal concentrations of reducing sugar (NG, G, GP-meal) and glucose (15%) start earlier than that of PEG, due to the later discussed relation between the completeness of absorption and the mean transit time.

The jejunal concentrations of fat and protein (Fig. 1) reflect the separation of the composite meals in the stomach, and minimal concentrations are found in samples from the second to fourth 20-minute periods. Toward the end of the experiments the concentrations of fat tend to increase, which mirrors the late arrival of the separated fat into the gastric antrum.

The gross pattern of absorption. As demonstrated in Figure 2, most of the meal carbohydrates are absorbed in the upper intestine within the first 80

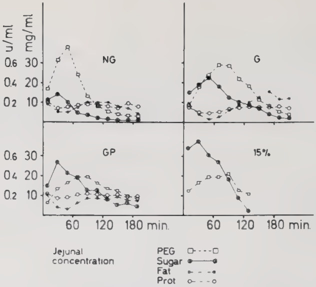

FIG. 1. Mean concentrations of test meal marker, PEG (units/ml), and meal constituents (mg/ml) in consecutive jejunal samples after the different meals. Concentration of PEG in test meals 0.7 units/ml.

minutes after intake of the meals. A considerable amount of test meal fat was absorbed late after ingestion, most marked for the G-meal, after which a higher amount was absorbed during the third compared to the first hour ($p < 0.05$). Only slight variations in the protein absorption was recorded after the first hour.

The absorption in the segment from the first hourly loads are given in Table I. Significantly higher amounts of fat and protein were absorbed from the NG-meal compared to from the glucose-containing meals (fat: NG vs G $p < 0.01$; vs GP $p < 0.02$; protein: NG vs G $p < 0.001$, vs GP $p < 0.01$), whereas higher amounts of carbohydrates were absorbed from the latter (G vs NG $p < 0.01$). These differences result from the different gastric emptying patterns of the meals.

Also evident from Table I and Figure 2 is the fact that comparatively large portions of the test meals pass unabsorbed at the sampling level during the early parts of the experiments to be propulsed to lower parts of the intestine. This applies particularly for carbohydrates after all glucose-containing meals and for fat and protein after the NG and GP-meals.

Absorption of carbohydrates in relation to loads. The amounts of sugar absorbed during transit were linearly related to the loads (Fig. 3 upper) over the range of loads recorded after all meals according to

$$A = bL + i \qquad \textit{Equation 1}$$

where A mg sugar was absorbed from the load, L mg, during transit of the proximal 70 cm of the intestine.

Although not significantly different, the absorptive efficiency of carbohydrates was higher after the G-meal than after any of the other meals (Table II).

Absorption of fat in relation to loads. Estimation of the net endogenous contribution. The absorbed amounts of fat were linearly related to the loads (Fig. 3 lower). Similar slopes of the regressions were obtained after the NG and

G-meal both when twin experiments and mean values (Table II) are compared, whereas the efficiency of fat absorption in the segment was somewhat lower after the GP-meal. The presence of negative intercepts in all individual regression equations (range 16 to -378, $p < 0.01$) implies a

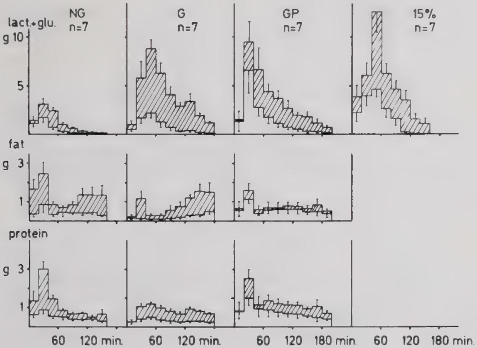

FIG. 2. Absorption of sugar, fat, and protein after the different meals. The higher staples represent the intestinal loads corresponding to each jejunal 20-minute period, i.e., the amounts that should pass at the sampling level if the absorption was nil (Mean ± S.E.M.). Unfilled staples are the amounts that passed the sampling site per 20 minutes (Mean ± S.E.M.). Hatched areas represent the amounts absorbed during transit of the upper 70 cm of the intestine. The absorption (A_n) from a load is expressed in g/70 cm/tm_n min and is desired from: $A_n = L_n - P_n$. L_n g is the intestinal load of a nutrient corresponding to the nth jejunal 20-minute period. L_n left the stomach concomitant to the nth jejunal portion of PEG on an average tm_n minutes earlier during $20 - \Delta tm_n$ min. L_n was emptied from the stomach at the average rate of $L_n/20 - \Delta tm_n$ g/min. P_n g is the amount of the nutrient, which passed unabsorbed at the jejunal sampling site during the nth 20-minute period. A_n/tm_n g/70 cm/min is the average absorption rate from L_n in the investigated segment. $P_n/L_n = 1 - A_n/L_n$ is the proportion of L_n not absorbed during transit.

TABLE I

Absorbed amounts of carbohydrates, fat, and protein (g) in the proximal 70 cm of the intestine from the loads (g) which had traveled the segment within 60 minutes after meal intake. Given in table are also the amounts which passed unabsorbed at the investigated jejunal level during the first hour (g). Mean ± S.E.M.

			Absorption (g)	Loads (g)	Passed amounts (g)
Sugar	NG	7	3.3 ± 0.7	6.9 ± 0.3	3.5 ± 1.4
	G	7	11.2 ± 1.8	15.7 ± 2.4	4.5 ± 1.8
	GP	6	8.5 ± 2.1	16.3 ± 3.9	7.8 ± 2.6
Glucose	15%	7	14.9 ± 2.6	25.6 ± 2.5	10.7 ± 4.4
Fat	NG	6	3.3 ± 0.5	4.9 ± 0.8	1.6 ± 0.7
	G	7	1.3 ± 0.4	1.7 ± 0.3	0.8 ± 0.3
	GP	5	1.1 ± 0.4	2.8 ± 1.4	2.3 ± 1.1
Protein	NG	6	3.6 ± 0.3	5.8 ± 0.4	2.1 ± 0.9
	G	7	1.3 ± 0.2	2.5 ± 0.2	1.1 ± 0.5
	GP	7	1.4 ± 0.6	5.3 ± 1.2	3.3 ± 1.3

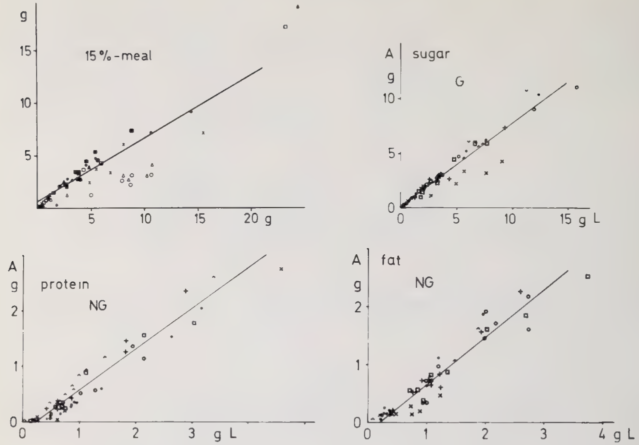

FIG. 3. Relations between the intestinal loads (L, g) and amounts absorbed during transit (A, g) for reducing sugar after the G-meal and for glucose after the 15 percent glucose solution (upper) and for fat and protein after the NG-meal (lower). The mean slopes of the individual regressions depicted.

negative absorption from loads lower than defined by the intersection of the regression line on the horizontal axis. Since negative absorption means secretion, a net addition of endogenous fat was suspected. In six experiments with the 15 percent glucose solution the concentrations of fat in the jejunal samples multiplied by the flow volumes equal the net amounts of added endogenous fat at the investigated jejunal level (Table III). Since the magnitude of the endogenous contribution is presumably related to the composition of the meal, these figures are not directly applicable to the conditions after composite meals. Bilirubin was chosen as a reference substance, since the main source of endogenous fat is bile. In six experiments with the glucose solution, the average amounts of fat (x, mg/20 min) and bilirubin (y, mg/20 min) were related according to the regression equation:

$$y = 0.053\,x - 0.40 \text{ (S.E. } (b) \text{ } 0.015,\ r + .832,\ n\text{:}7)$$

The average amounts of endogenous fat after the composite meals were estimated from this relation (Table III) and the average fat absorption figures corrected for the dilution (Table II).

The absorption of protein in relation to loads. Estimation of the net endogenous contribution. The linearity of the relation between the loads and absorbed amounts is demonstrated in Fig. 3 and Table II. Negative intercepts were obtained in all but two experiments, indicative of a secretion of protein added in the stomach and intestine and not absorbed during transit of the upper intestine.

In five of six experiments with the glucose solution, significant linear regressions were obtained between the amounts of protein (Table III) and lipase at the jejunal sampling site (mean r + .906 n:5). The average amounts of protein (x, mg/20 min) in six consecutive 20-minute periods were related to lipase (y, u/20 min):

$$y = 8.25\, x - 1253 \text{ (S.E. } (b) \text{ 0.68, } r + .987\ n\text{:6)}$$

The average net contribution during three hours amounted to 4.7 g after the GP-meal and to 3.2 and 2.8 g respectively after the NG and G-meals (Table III). After correction for the endogenous dilution, only minute amounts of the

TABLE II

Regressions between loads (x, *mg*) *and amounts absorbed during transit* (y, *mg*) *were calculated for individual experiments. Mean values for the slopes of the regressions* (b) *are given* ± *S.E.M.*$_i$ *between (e.g., inter) individuals and estimation S.E.M.* (b)$_w$ *within the individual regressions, i.e., the pooled variances of* b *divided by the number of experiments. Mean correlation coefficient* r. b$_{corr}$ *is the slope of the regression after correction for the endogenous net contribution of fat and protein and was calculated from mean values for 20-minute periods.*

	meal	n	b, mean ± S.E.M.$_i$	S.E.M.$_w$	r, mean	b_{corr}	n	r
Sugar	NG	7	+.598 ± .086	.060	.950			
	G	7	+.753 ± .063	.035	.982			
	GP	6	+.589 ± .114	.031	.962			
	15%	7	+.585 ± .084	.031	.981			
Fat	NG	6	+.825 ± 0.78	.092	.965	+.792	9	.971
	G	7	+.840 ± .088	.057	.976	+.773	9	.972
	GP	5	+.613 ± .306	.151	.768	+.696	10	.921
Protein	NG	6	+.741 ± .037	.050	.977	+.823	9	.997
	G	7	+.731 ± .052	.091	.935	+.832	11	.977
	GP	7	+.641 ± .062	.118	.886	+.744	10	.931

TABLE III

Average net contribution of endogenous fat and protein at an intestinal level of 130–140 cm from the mouth (g/hour).

		Hour		
		1st	2nd	3rd
Fat (g)	NG	0.644	0.572	0.306
	G	0.400	0.263	0.266
	GP	1.360	0.610	0.512
	15%	0.619 ± 0.277	0.284 ± 0.127	
Protein (g)	NG	1.178	1.126	0.891
	G	1.004	0.950	0.816
	GP	1.770	1.510	1.435
	15%	1.262 ± 0.564	0.690 ± 0.309	

dietary protein were found to be present in the jejunal contents at the investigated intestinal level, except for periods with very high loads.

The absorption of carbohydrates in relation to the mean transit time. Provided that the absorptive capacity of an intestinal segment is not exceeded, a strict linear relationship between the loads and the absorbed amounts could be expected, when the length of the segment and the time during which the absorption takes place are fixed. As previously shown these times, i.e., the transit times, vary during the course of the experiments. It was, therefore, suspected that the deviations from a strict linearity in the regression equations according to Equation 1 and the positive intercepts invariably found in the equations (G and 15% $p < 0.05$) were caused by variations of the mean transit time. An analysis was made of the experiments with the G-meal and the 15 percent glucose solution and the plot of the experimental data (Fig. 4) supported the reasoning.

The nonabsorbed fraction of L $(1 - A/L)$ decreased with an increasing mean transit time (tm, min) according to:

$$\log (1 - A/L) = \log i - K\, tm$$

values from ten to twelve 20-minute periods from each G-experiment were used to calculate individual regressions. The mean value of K in the seven experiments was 0.938 ± 0.007, the mean intercept was 0.689 range 0.439 – 1.026 and the mean correlation coefficient was −0.718.

After the 15 percent glucose solution values from six to ten 20-minute periods were used. The mean value of K was 0.907 ± 0.037, the mean intercept 0.934 range 0.595 – 1.504 and the mean correlation coefficient −0.796.

The quantitative importance of tm has so far been tested in the series with the G-meal. $1 - A/L$ is equal to P/L where P (mg) pass the jejunal sampling site during a 20-minute period and is the nonabsorbed part of the load L (mg). A significant decrease of residual mean squares was obtained in six of seven experiments by insertion of tm as a second independent variable together with $\log L$ in multiple regressions against $\log P$. Since no elimination of sugar occurs from the intestinal lumen except by absorption, $\log P$ may be substituted against $\log A$. The high correlation coefficients obtained in regressions

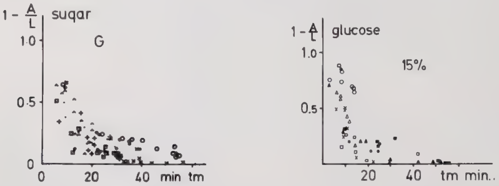

FIG. 4. Plots of the mean transit times (tm, min) against the nonabsorbed part of the sugar $(1 - A/L)$, i.e., the part of the loads which leave the 70 cm segment unabsorbed in 7 experiments with the G-meal and 7 experiments with the 15 percent glucose solution.

between log *L* and log *A* (mean *r* + 0.982) increased, when *tm* was inserted as a second independent variable together with log *L* against log *A* in multiple regressions. A stepwise procedure demonstrated a significant decrease of the residual mean squares in all experiments. The mean correlation coefficient for the seven multiple regressions was + 0.997.

Absorption rate in the segment. The average absorption rate during transit of the loads may be determined by dividing the absorbed amounts with the corresponding *tm* minutes.

The highest recorded average absorption rate of carbohydrates was 785 ± 191 mg/min per 70 cm from the third intestinal 20-minute load after the 15% glucose solution. Individual peak absorption rates occurred correspondent to the second to fourth period. Mean of the highest individual rates was 842 ± 197 mg/min/70 cm per 20-minute load.

The absorption rate of fat and protein was calculated by dividing the average absorbed amounts after correction for the endogenous contribution with the average mean transit times. The highest rates were recorded early after the NG-meal amounting to 259 and 185 mg/min per 70 cm and 20-minute load for fat and protein respectively. Uncorrected figures for maximal rates in the separate NG-experiments were for fat 326 ± 78 mg/min and for protein 255 ± 69 mg/min. The corresponding figure for protein after the GP-meal was 145 ± 39 mg/min/70 cm.

Plotting the mean absorption rates after the NG and G-meal against the mean gastric emptying rates both expressed in kcal per minutes (Fig. 5) demonstrates that the two meals follow the same curvilinear relation (depicted according to text in figure). It is suggested from the shape of the curve that the absorption rate in the segment was limited by the gastric delivery rate of calories.

Discussion

The importance of the composition and physical properties of a meal in determining its intragastric behavior, its emptying and subsequent absorption is evident from the early absorptive patterns after the different meals.

The early divergent gastric emptying pattern of the carbohydrate-poor NG-meal compared to of the glucose containing composite meals resulted in

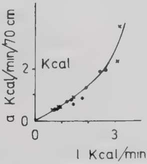

FIG. 5. Average gastric emptying rate of calories (1, kcal/min) plotted against the average absorption rate (*a*, kcal/min/70 cm) after the NG and G-meal. The best adaptation to a straight line relation was obtained from: $1/a = 1/1:b - i$. Note that approximately the same relation was obtained after the two meals (NG *b* 2.22, *i* 3860.51, *r* 0.83, *n*:8; G *b* 2.27, *i* 3470.92, *r* 0.89, *n*:9).

corresponding dissimilarities regarding the amounts of the different components absorbed in the proximal intestine. Significantly larger amounts of fat and protein were absorbed early after the NG-meal whereas sugar dominated the early absorption after the glucose-containing composite meals.

The average figures for the absorption of sugar and fat, as estimated from the mean slopes of the regressions between the loads and the absorbed amounts in the present series, are consistent with those recorded by Borgström et al (1) at the same intestinal level after feeding a meal of essentially the same composition as the G-meal. They calculated the percentage absorption from the relation between the concentrations of a nutrient to PEG in the test meal and the same relationship in samples of intestinal juice at different levels. The validity of the method to determine the absorption of fat was later questioned (3), because of the intragastric separation of fat from marker. Applying this method to the data in the present series resulted in a very high percentage absorption of fat during the first hour after ingestion, declining thereafter with low or negative figures in later sampling periods. These systematic errors should be counterbalanced if the sampling period of chyme is extended to several hours or if the tube is allowed to pass down the intestine during the passage of the meal. They could be eliminated by inserting gastric concentrations of fat and PEG correspondent in time to the jejunal, i.e., the concentrations prevailing *tm* minutes ahead of each jejunal 20-minute sampling period. This confirms the observation (4) that the absorption of fat can be assessed from a water soluble marker, provided that the gastric quotients represent the emptied contents and correspond in time to the jejunal.

Although to a lesser degree than fat, the protein dissociated from PEG in the stomach. Another reason for the lack of parallelism between the gastric concentrations of protein and PEG is the gastric secretion of endogenous protein. To the somewhat lower average absorption of protein in Borgström's series, their use of radioiodinated albumin to indicate the dietary protein may also have contributed. Later studies have demonstrated the recirculation of radioiodine (5), which makes it a less suitable marker.

During transit of a meal in the upper intestine the dietary protein and fat are diluted by an endogenous addition. Previous estimates of the endogenous protein after food intake in man are scarce and vary considerably. Whereas the total contribution can not be assessed by the present method, the net endogenous protein at the jejunal sampling level can be estimated and corrected for in calculation of the absorption.

The net amounts of protein and fat at the jejunal sampling level were determined after feeding the protein free 15 percent glucose solution. These figures were not considered directly applicable to the conditions after composite meals, since the magnitude of the endogenous dilution is presumably related to the composition of a meal. By means of linear regression analysis lipase was found to be the test predictor of the endogenous protein, the error of the estimation being less than ±8 percent per 20 minutes. The

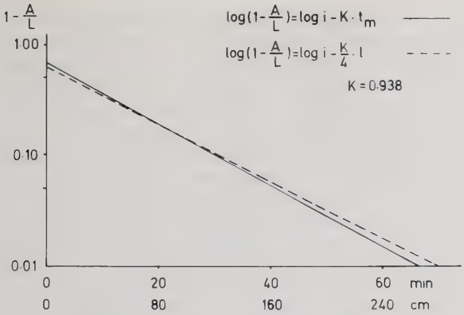

FIG. 6. Comparison of the mean slope of the individual regressions from date in Figure 4, G-meal and the slope for the relation between length of the intestine and the nonabsorbed fraction of sugar calculated from the data by Borgström et al (1); see text.

average net endogenous amount of protein after the composite meals amounted to 2.8 g, 3.2 g and 4.7 g during three hours, i.e., are within the lower part of the range (2–8 g) estimated by Nixon and Mawer (6) after a low-protein, fat-rich meal at levels 110–140 cm from the mouth.

Bilirubin was chosen as an indicator of the endogenous fat, of which the main contributor is bile (7). The average net addition after the composite meals did not exceed 5 g in three hours.

One of the variables involved in the process of intestinal absorption seems to have been little recognized, namely the time during which the absorption takes place, i.e., the transit time through an investigated segment. In studies with segmental perfusion techniques (8, 9) the absorption rate is derived from dividing the absorbed amount by the sampling time, which expresses the absorption from the per minute load infused. The length of the sampling period is evidently not related to the time during which an infused test substance is exposed to the mucosal area of the segment. Consequently, comparisons of the absorption rates of different test substances or of the same test substance at different infusion rates or concentrations may show differences, which are caused by different transit times through the segment and are not related to the absorbability per se.

In the present study the absorption rate was determined from dividing the absorbed amount by the corresponding mean transit time, and expresses the average rate during transit of the 70-cm segment by a 20-minute load. The absorption rate thus calculated may be divided by the sampling time, which is constant and experimentally controlled to provide a figure for the per minute absorption from the per minute intestinal load or by the gastric emptying time, $20 - \Delta tm$, to express the per minute absorption from the per minute delivery. The obtained absorption rate is representative for every minute of transit and, if divided by 70, for every cm of the segment only when the maximal absorptive capacity is reached or exceeded. The latter was not the case in the present series. In the absence of the absorption integral through the segment the rate can be plotted against the experimentally elapsed time or, as shown in Figure 5, against the corresponding gastric emptying rate.

The highest absorption rates for all meal components were recorded within the first hour after ingestion of the meals (resulting both from high loads and short mean transit times). For sugar the mean of the individual maxima in this series corresponds to an absorption rate of 12.0 ± 2.8 mg/min/cm which lies between the V_{max} estimated by Holdsworth and Dawson (8) and Modigliani and Bernier (9) with perfusion techniques. No conclusions can be drawn from our obtained maximal absorption rates of fat and protein since comparative figures are lacking.

The maximal absorption capacity of the 70-cm intestinal segment was not exceeded for any of the components after either meal, as indicated from the straight line relationship between the loads and the amounts absorbed in the segment. This can evidently be brought about by two adaptive mechanisms, namely, by regulation of the gastric emptying rate or by prolonging the time of contact between the products of digestion and the absorbing surface in the segment. By either of these or a combination of the two an absorption efficiency according to the slopes of the regression lines (Table II) may be kept.

The magnitude of the load was quantitatively the most important determinant of the absorption of sugar after the G-meal. The influence of the mean transit time, however, was found to be significant when tested by a stepwise procedure in multiple linear regressions.

When a maximal absorptive capacity of a segment is reached, the load should decrease linearly during transit of the segment, due to removal of a constant amount per time by absorption. Should the length of the segment or the transit time increase, the load should decrease linearly until saturation conditions are no longer fulfilled, after which the load should decrease along a semilogarithmically linear function due to the removal of a constant fraction per time. The latter of these functions could be defined by the present data. After the 15 percent glucose solution the absorbed part of the glucose at the sampling level increased by an average fractional rate of 9.3 percent per minute increase of the transit time through the segment and after the G-meal the corresponding figure for sugar was 6.2 percent. Suggested from the intercepts in the regression equations the elimination rates are higher at very short transit times than might be predicted from the continued course of the curves. Although it is probable that these parts of the relations ought to be described in terms of saturation kinetics, the data from the present series were inadequate for such a characterization. The shape of the relation does, however, inform us of the serious consequences of a rapid transit on the absorption.

The similarity of the plot in Figure 4 for the G-meal to that obtained for the same meal by Borgström et al (1) for the length of the intestine against the percentage absorption prompted a calculation of their figures. The absorbed part of the sugar was thereby found to increase by 1.5 percent per cm increase of the intestinal length from the pyloric sphincter (K 0.985, intercept 0.620, r – 0.744, n:80). The analogy between the length of the intestine and the transit time as to the percentage elimination of a nutrient from the intestinal lumen (Fig. 7) is being further investigated. It permits the construction of a relation between the velocity at which the intestinal contents are propelled and the length of the intestine needed for complete absorption. From such a relation the time course of the absorption in the entire intestine may be estimated.

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X. Human Gastric and Intestinal Response to Meals: General Discussion

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Although at present a large number of gastrointestinal functions and factors influencing these functions are known, little can be concluded as to their quantitative importance in normal digestion and absorption in man.

Physiological experiments in which the conditions are simplified to analyze the effect on one variable of changes of one or several parameters usually give clearcut answers. However, the setup of such experiments has often hindered physiological mechanisms from operating, a typical example being the determination of the gastric secretion by withdrawal of the secreted juice. Therefore, to study the normal physiology of the human gastrointestinal tract, we tried to disturb as little as possible normal ingestion, gastric storing and mixing and intestinal mixing, transit and absorption. Our experimental conditions have so far been successful, as only smaller portions of the ingested food are removed at the sampling site 70 cm from the pyloric sphincter and hence prevented from further contact with the intestinal mucosa.

An evident inconvenience of the method is that intubation is required. This procedure could possibly add a nonphysiological vagal stimulation. We had to navigate between Scylla and Charybdis. One more tube in the duodenum would make it possible to measure directly the duodenal secretion and the volume of contents in the upper intestine. It would, however, have transformed the procedure from a rather convenient to an unpleasant one for the studied persons with evident effects on the results. The experimentally found even gastric emptying rate after the G-meal indicates that the intubation did not appreciably affect the results. Another indication is that the total evacuation time of the G-meal in our experiments is similar to that recorded by Mattsson et al (1) in x-ray studies of the same meal mixed with barium sulphate.

The method introduced by us permits the measurement of the intestinal mean transit time and its variations. It also permits quantitation of the fluid volumes at a jejunal level, hitherto never measured in man. These variables are necessary for calculation of gastric events from jejunal data and they are required for a correct understanding of the dynamics of the intestinal transport and absorption.

Our meals are relatively small as regards volume and caloric contents. This is an advantage when analyzing the effect of stimulating or inhibiting factors as maximal effects are obtained only during short times. They differ in this respect from the meals used in the study by Brunner, Northfield, Hofmann, Go, and Summerskill (2). Although the technique used by them does not allow any detailed study of osmotic effects or effects of different food constituents, their results convincingly show that maximal stimulation of pancreatic enzymes occurred for most of the day. Not until six hours after the last meal did the enzymes begin to fall. This result is in accordance with our finding of an initial maximal stimulation of the pancreatic enzyme secretion, which falls when the intestinal load of food constituents decreases.

To assure the readers of the necessity for our use of the rather complicated methods of calculation to get reliable values of mean transit time, flow and mixing, it may be informative to give some steps in the development of the method through the last ten years.

During the first work with the multiple indicator method (3), it was found that the jejunal flow rates calculated from single or total indicators were not equal. Since they should be equal, if the mean transit time was constant, the discrepancies were evidently due to changes in mean transit time that later could be estimated for single periods from the slope of the 100 c/C curves according to principles given by Comet and Lagerlöf (4). In searching for the relation between flow and mean transit time, tm, Equation 10 from Chapter II was found already in an early stage. Since the determination of the slope of the curves did not give exact values, a search was made for an unbiased arithmetic method and many variants were tested until the procedure now used had been worked out. The reliability of the theoretical mathematical background is now evidenced by the fact that a sufficient number of equations may be obtained to calculate the unknown jejunal fluid flow. The practical applicability was earlier illustrated by the good correspondence between found and calculated values in the model experiments. Although as a result of the long development time some differences may be found in the present monograph and preceding original articles, there are relatively few.

Against the analysis of indicator concentration curves given in Chapter II it may be argued that the exponential time-frequency distribution proposed by Zierler (5) presupposes either a single mixing chamber or, as may be understood from the analysis of circulatory indicator concentration curves by Comet and Lagerlöf, a row of mixing chambers with the same relation between volume and flow. Neither situation is strictly correct as seen from the feeble converted S-shape of the $(100 \cdot c/C)/10$ curve in Figure 4 of Chapter II. It must be remembered, however, that in the practical work the curve analysis is used only for extrapolations regarding parts of the last two indicators and that the importance for the calculations of exact extrapolations is small.

Some hesitations may be evoked by our use in the practical work of Equation 10, Chapter II to calculate tm for the first two jejunal periods before the initial contents are washed out. The argument would be that if we accept the logarithmic time-frequency distribution of indicator, the tm's should be somewhat too high. Even with the given presumptions, the error will be annihilated, however, when 20-minute periods are used to describe gastric emptying or bile and enzyme output. $\Sigma\ \Delta\ tm$ and tm_t from Equation 5 of Chapter II are equal when the initial duodenal-jejunal contents have been washed out. With the tm_t prevailing, the jejunal indicator flow before this time will always be put back to the first duodenal period where it belongs, regardless of how *tm* is calculated.

In the calculation of *tm* it was assumed that the *tm* for duodenally infused indicator and PEG from the stomach were equal. This is not strictly true, since the rate of input of PEG in contrast to that of duodenal indicator varies. The error is insignificant, when single periods are concerned.

One of the relations most difficult to comprehend for us and probably also for the reader has been the relation between mean transit time, *Tm*, and mean passage time, *Pm*, for an indicator at the sampling site. It is probably best understood if we start with the conditions at the infusion site in the duodenum. Here the indicators are swept away by the emptied gastric contents and the duodenal secretion, for which reason the transit time is zero. The passage time for 200 units of indicator is 40 minutes. Since the infusion is constant, the first particles pass at time zero and the last at time 40 minutes. The mean passage time is therefore 20 minutes. We observe that the mean passage time of 200 units is the passage time of 200/2 units and that the passage time of any amount of indicator will be proportional to the amount infused.

It is evident that, whatever the time distribution of flow times concentration of single indicator in jejunum, during constant conditions the passage time must be proportional to the corresponding amount of indicator infused. We therefore have to investigate how long a time an amount of indicator equal to the infused, i.e., 200 units, would take to pass the sampling site if this amount of indicator was equally distributed in time. By calculating *Pm* according to Equation 8 of Chapter II, we obtain half this time, which is accordingly the passage time for 100 units that should be used in the calculation of mixing according to Equation 7 of Chapter III.

Since *tm* can be calculated by an unbiased mathematical method and *Pm* may be calculated from *tm* if the appearance time, *a*, is known, it would be of value to know the relation between *a* and *tm*. It would then be possible to calculate *pm*'s also for single periods directly from their *tm*'s. Two possibilities exist. During transient changes of intestinal volume, the *tm*'s of all frequencies of indicator concentrations at the sampling site originating from different times are apt to increase or decrease to the same extent. The appearance time then increases with $\Delta\ tm$ as shown in Chapter II, Fig. 8, but *pm* does not change. During more prolonged changes of volume, *a* and *pm* may change proportionally to *tm*. By relating *a* to *tm* in cases with long *tm*'s we tried to investigate which alternative was dominant. Unfortunately, the appearance time was equally well correlated to $\Delta\ tm$ and *tm*. Because of the shortcomings in our determination of *a*, we have at present postponed the trial to calculate *pm* for separate periods, although preliminary calculations from mean values indicate specific patterns for the different meals to be present.

Gastric secretion

Cephalic phase. In contrast to normal food our fluid meals are not chewed. The drinking time is short and the meals not exceptionally tasty, wherefore the central vagal stimulation should be weak and of short duration. This conclusion is corroborated by sham feeding experiments in man by Janowitz et al (6), who found no increase of the basal secretion after a gruel meal, but observed a secretion of 10 mEq per hour after a meal selected by the patient and containing meat and vegetables.

Gastric phase. Since the gastric phase should be mainly due to the release of gastrin from the gastric antrum, it is of interest to analyse the conditions for gastrin liberation.

The release of gastrin is inhibited when the antral pH is below 3 (7). After the composite meals in our series the antral pH fell to 3 or less within 90 minutes after ingestion. From this may be concluded that antral liberation of gastrin from central vagal stimulation, distension of the stomach or from protein hydrolysate could stimulate the gastric secretion only during the first 90 minutes. Nevertheless, gastric secretion continued at an almost constant rate as long as the meals remained in the stomach. Preliminary results of C. Johansson and G. Nilsson demonstrate a moderately increased and constant serum level of gastrin throughout the entire GP-experiments, indicative of an extragastric source of the released gastrin.

Intestinal phase. In accordance with what was said about the cephalic and gastric stimulation of gastric secretion, we may assume that the secretion at least during the second and third hour is regulated from the intestine. The balance between stimulatory and inhibitory factors is such that the gastric secretion proceeds at an approximately constant rate as long as the meals are emptied from the stomach. After the completion of the initial and adaptive phases of the gastric emptying, the stomach delivers both contents and calories to the intestine at a constant rate, the caloric transfer per time being almost equal after all composite meals. The stimulus to intestinal receptors which by their activation should influence the continued gastric secretion is therefore kept at an even level.

Compared to the feeble and relatively short-lasting gastric secretory response after the glucose solution, a higher secretion was recorded after the composite meals. Since the secretion rate was almost equal after all composite meals, the different total secretory volumes result from a shorter or longer-lasting stimulation which, in turn, should be due to different evacuation times of the meals from the stomach.

Having suggested that emptied meal components have a stimulatory effect on the continued gastric secretion, it is possible to hypothesize on their mode of action. The cells liberating gastrointestinal hormones have microvilli on their intestinal surface, indicating absorption of elementary food components to be of importance for their release of gastrohormones. But they are also richly innervated, making them able to partake in afferent and efferent reflexes, the latter stimulated by absorbed food constituents or by receptors located in other places. If so, rapid changes would be mediated by reflexes, slower changes by the release of hormones, whose accumulation in the blood is governed by the rate of liberation and disappearance. It is in this respect necessary to remember that the half-life of hormones studied with isotopes or with radioimmunologic methods usually does not correspond to the half-life of the biologic effect produced.

As regards the intestinal phase of gastric secretion in the first place, duodenal gastrin could seem responsible. It is distributed along the duodenum (8, 9) and may be liberated by amino acids at the pH prevailing in the duodenum. A second operator might be cholecystokinin, known to be released both by fat and protein. It may be stressed that at present a quantitative evaluation of the importance of different factors is impossible. From an evolutional point of view a cooperation between gastrin, cholecystokinin, and vagal reflexes would be most tenable.

Because of the rather uniform behavior of the gastric secretion during the composite meals, any definite inhibiting properties of the different food components cannot be distinguished. Grossman has shown secretin to have strong properties in this respect (10). That the amount of duodenal fluid secreted after G-meal amounts to the same order of magnitude as the gastric secretion (vide infra) indicates a strong liberation of secretin-like substances. In this connection it must be remembered that acid reaction is no prerequisite for secretin release. Secretin-liberating cells are spread through a great part of the small intestine, where the pH lies around 6.5. It was shown already in 1924 by Mellanby (11) that bile salts have the property of stimulating a secretin-like pancreatic juice, a fact confirmed in man by Lagerlöf in 1942 (12). Since glucagon is chemically related to secretin, it could be another inhibiting hormone. Wormsley's demonstration of a high pancreatic secretion after intrajejunal administration of acid is an important evidence of the wide spread of hormones with secretin-like properties (13).

Acid concentration in gastric juice. Since gastric juice, expecially at the antral level, is composed of components from several cells, it would not be surprising if its acidity varied. From experiments analyzed by Schütz (14) it is known that the total water flux is much higher than the net water flux. Under such conditions osmotic influences cannot be disregarded; the average low acidity of the gastric juice produced in the beginning after the 15 G and G-meals point in this direction, although differences in the stimulation of the antral glands may be equally important. A hint in this direction is a constant acid concentration in the gastric juice secreted after the glucose solution. The acidity after the NG-meal, where osmotic influences are small, is very similar in all cases, lying around 110 mEq/1. The presence of a buffering meal here excludes back-diffusion of hydrogen as a course of decrease of the theoretical initial hydrochloric acid value proposed by Teorell, 177 mEq (15).

Gastric emptying

Effect of gastric separation. Because of the separation of food constituents in the stomach it is necessary to describe the emptying of different components of the gastric contents separately, if one aims at an analysis of the mechanisms involved. Although the motor activity leading to gastric emptying is a complex procedure, the many works of Hunt and his associates (16) have shown the usefulness of the emptying rate as a measure of the gastropyloric motor activity. The direct measure of this activity is then the volume of gastric contents emptied, not the amount of marker, a point often neglected in the literature.

Effects of food constituents. After the glucose-containing meals the gastric emptying rate was promptly inhibited. After the glucose-free NG-meal the inhibition of gastric emptying was a slow process as evidenced by the higher rate of emptying during the first 20 minutes compared to the G-meal. It could be due to a decrease of the propulsive forces since uninhibited emptying is positively related to gastric volume. The other factor responsible for the inhibition of gastric emptying would be the presence of digested fat in the upper intestine, the effect of which was already shown by Shay in 1934 (17) and in more detail by Hunt and collaborators (18, 19). This factor ought to be more prominent after the NG-meal than after the other meals due to the higher initial delivery rate of fat, the emptied fat being rapidly spread over and activating a large area of the intestine.

During the emptying of the hypertonic glucose-containing meals the glucose dominates the inhibition during two to four 20-minute periods. After a short period of imbalance during which hyperosmotic material is rapidly spread through the intestine, the emptying is adjusted to protect the intestine for inconvenient osmolarity. A given amount of surplus osmoles is then emptied per minute. As the meals are successively diluted in the stomach, the volume emptied increases. The rise in acidity of the gastric content may contribute as its neutralization by duodenal bicarbonate leads to a decrease of ions presumably without changing the duodenal pH (1).

When the dilution of gastric contents in the duodenum has reached a certain level the inhibition caused by fat prevails; and all fat-containing meals behave similarly. Protein at the concentrations used does not add further to the inhibition since after the GP-meal the rate of emptying was not decreased. During this stage fat is delivered at a constant rate to the intestine after all composite meals. After the NG-meal, this phase follows as soon as a full inhibition is established. At this time both fat and protein are delivered to the intestine at constant rates.

The 15 percent glucose solution behaves, surprisingly enough, similarly to the meals containing glucose after the first period of constant delivery of glucose, although we would expect less inhibition of the emptying. The explanation would be that the gastric propulsion is decreased due to less gastric secretion and volume. Combined with less inhibition by cholecystokinin, evidenced by marked decrease of bile flow, the net result would be constant emptying leading to a further decrease of the volume of gastric contents and a rapid decrease of the volume after the first two hours. This pattern is in accordance with our experimental findings.

Possible mediators of observed effects. To speculate about the importance of different mediators in the complicated emptying process may seem futile as the knowledge of the multipotence of gastrointestinal hormones increases continuously as well as the number of isolated hormones. A new field is also the effect of prostaglandins (21, 22).

As a base for further studies some notions may be warranted, however. For reasons discussed for gastric secretion it is unlikely that central vagal impulses have a strong influence on the course of emptying. The rapid inhibition of the gastric emptying after feeding due to concentrated glucose could be due to the release of secretin as a substantial rise in serum secretin was recorded within five minutes after a peroral load of glucose by Young and Chisholm (23).

To separate the effects of vagal reflexes from the effects of liberated cholecystokinin is impossible, especially since the neural stimuli and the liberation of cholecystokinin may both be caused by absorption of substances in the same cell. Both are known to contract the gall bladder and relax the sphincter of Oddi, vagal impulses to increase antral motility. The effect of cholecystokinin in this respect is not sufficiently studied.

Intestinal water balance. As shown in 1944 by Vischer et al, the in- and outflow of water through the intestinal mucosa is much higher than the absorbed or secreted amounts (24). Similar conditions were found by Lagerlöf et al (20) in human duodenum. Whatever method used to determine secretion of water into or absorption from the intestine, it must be remembered that only the net secretion or the net absorption can be measured if the water is not indicated.

The duodenal water balance. The duodenal concentrations of PEG after the same meal as our G-meal have been determined by Fields and Duthie (25). By dividing our mean values for gastric emptying of PEG with their corresponding mean concentrations, the duodenal flows after the G-meal may be roughly estimated. If the obtained figures are plotted together with our figures for gastric emptying of water and jejunal flow and changes in *tm* are considered, the relations illustrated in Figure 1 are obtained. They may be used to get a general picture of the net water flux in the upper intestine.

Gastric emptying after a few minutes results in a duodenal net secretion of about 5 ml/min. It reaches a maximum of 6.5 ml/min after 20 minutes. The increase of flow and its magnitude tallies well with figures for duodenal flow, obtained by Schütz, Andersson, and Lagerlöf (26) after continuous infusion of high doses of secretin and cholecystokinin. The absorption of water in duodenum should, therefore, be small during this period, presumably because of the high osmotic pressure of the duodenal contents and the low mean transit time prevailing at this time.

As the concentration of glucose in the duodenum successively diminishes due to dilution with gastric and duodenal secretions, the stimulation of gall bladder emptying and pancreatic secretion decreases. At the same time the osmotic forces retaining water in the duodenum decrease. As a result the net duodenal secretion decreases to reach zero values after about 80 minutes, thus indicating that absorption is balancing secretion at this time.

The jejunal water balance. When the duodenal secretion has reached its maximum the gastric contents already diluted with gastric secretion are

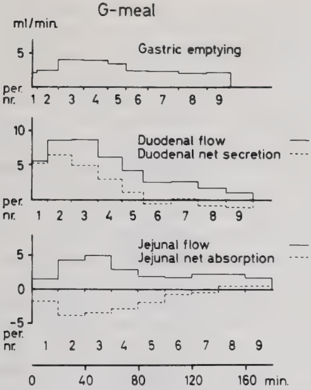

FIG. 1. Water balance in the upper intestine after the G-meal calculated by combining our mean figures for gastric emptying and jejunal flow with duodenal concentrations of PEG from the work of Fields and Duthie. Note the delay in maximum flow rates at lower levels.

further diluted in the duodenum by two or three times. As a result the contents leaving the duodenum should be isotonic after around 30 minutes. At this time about 120 ml isotonic fluid reach the upper 30 cm of jejunum per 20-minute period and is absorbed to about 60 percent. As the jejunal load after a further 20 minutes rapidly drops due to increased net duodenal absorption, the net jejunal absorption decreases to insignificance at the same time as the jejunal flow keeps constant. Intestinal secretion seems to balance absorption.

The pattern of interactions proposed above should, in its main features, be relevant also for the GP and 15 percent glucose solution. The difference for the GP-meal should be a stronger and longer acting stimulus to the gastric and pancreatic secretion resulting in somewhat higher and longer standing jejunal rate of flow. After the glucose solution stimulation is weaker and less sustained.

The volume of pancreatic, biliary, and intestinal secretion after the NG-meal cannot be assessed from available data. But the presence of gallbladder emptying and of protein split products indicate stimulation via release of cholecystokinin and secretin.

Intestinal transit. The intestinal mean transit time may be used to estimate the pressure, P, necessary to accelerate the intestinal fluid to the prevailing velocities according to $P = V^2/2 \cdot 981$, where V is the velocity in cm/second and 981 the acceleration of gravity in cm/second. For a representative flow of 100 ml per 20 minutes and a *tm* of 10 minutes in the beginning of an experiment the velocity is, for example, $70/10 \cdot 60$ cm per second. P then amounts to only 0.025 g per cm^2. In comparison to the pressures measured in the intestine this pressure is negligible. It illustrates the fact that the main motor function of the intestine is to afford multiple high resistances, varying in location, necessary for the retention of chyme sufficiently long for adequate mixing and absorption.

The main patterns of intestinal motor actions are described as rhythmic segmentation and peristaltic rushes. The result of the first is directly measured in our mixing index which measures the delay of mean transit time due to mixing. The latter are illustrated from the downward irregular slopes of the changing $\underline{tm}$. To these functions is added the maintenance of the tone of the intestine necessary to regulate the capacity.

Our model experiments have illustrated how variations of input and capacity of the intestine together may operate to change jejunal flows and mean transit times. In the individual human experiments undulating changes of these variables have been seen indicating peristaltic rushes. In the mean figures these variations are obscured and the transit times after the first hour increase with time for all meals. The increasing transit time corresponds to a diminishing cross-section of the intestine at the sampling level (from cross-section $= (tm \cdot f/70 \cdot 20))$, indicating that the volume of the upper intestine decreases.

The fact that the jejunal flow and mean transit time after the NG and G-meal differ little in spite of great differences in the input from the stomach shows that tm and flow cannot be deduced from gastric emptying. It stresses the importance of net secretion or absorption for both these variables.

The lower mean transit times after the GP than after the G-meal are in accordance with the relation between volume, input and mean transit time found by Dillard, Eastman, and Fordtran (27). By use of a non-absorbed solution of the probably inert mannitole in constant infusion experiments, these authors studied the relation between volume load and mean transit time. Whereas at low perfusion rates the intestine adapts its capacity to the prevailing input, keeping the mean transit time constant, *tm* decreased at higher perfusion rates equal to those existing during the first hour after our meals. The main cause for the lower *tm*'s after the GP-meal than after the G-meal would be a higher duodeno-jejunal secretion indicated by the higher net duodeno-jejunal secretion and caused by the higher protein load.

The low transit time after the 15 percent glucose solution is explained by the high rate of gastric emptying due to the absence of fat-mediated inhibition.

As stated below the lower mean transit time during high loads induces a purposive employment of lower segments in the absorption.

Rate of indicator flow in lower intestinal segments. Although our experimental data only give quantitative information about the conditions in the upper part of the small intestine, they may be used in extrapolations to get a picture of the situation in lower intestinal segments. To make the extrapolations, assumptions must be made regarding the relation between the velocity of indicator particles in upper and lower intestinal segments. The assumption of a constant velocity of every indicator particle during its transit of the different segments was used, since the figures obtained with this assumption corresponded well with the results of Mattsson, Perman, and Lagerlöf (1). They investigated fluoroscopically the intestinal transit of the G-meal mixed with a barium sulphate suspension, which travels with the same velocity as PEG.

To understand the calculations it is necessary to remember that the transit of the total indicator is the same as that of a hypothetical indicator in which every indicator particle passes the intestine unmixed. The transit of indicator particles may be depicted in a coordinate system (Fig. 2) in which time is both abscissa and ordinate. The indicator particles arrive at the jejunal sampling site at a distance of 70 cm from their infusion site after times determined by their *tm* and reach 140, 280 and 350 cm after 2, 3, 4, and 5 *tm*, respectively. Figure 2 demonstrates, that an increase of *tm* with Δ *tm* leads to an increase of the time it takes the particles infused during 20 minutes, i.e., 100 indicator units, to pass a certain level. The increase will be one Δ *tm* per 70 cm. The flow of particles per 20 minutes will decrease accordingly.

The situation is most pronounced during the first 20 minutes, when Δ *tm* is high and consequently some particles advance much slower than others. During later peiods Δ *tm* is less, leading to more equal rates of flow for the indicator at high and low levels of the intestine.

The amounts of indicator passing per 20 minutes at different levels and times may be obtained from the figure. The amounts of total indicator contained in each intestinal segment at different times are shown in the lower

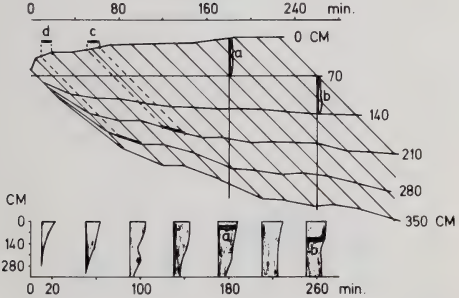

FIG. 2. Transit of the intraduodenally infused indicator through the intestine. The graph was constructed from the average amounts of indicator passing the sampling site every 20-minute period after the G-meals. The same time-scale is used on the abscissa and, although not depicted, on the ordinate. Hence the indicator particles travel in a direction of 45 degrees from the abscissa. The particles leave the infusion site at 0 cm and arrive at 70 cm, i.e., the sampling site after 1 *tm*, at 140 cm after 2 *tm* and at 350 cm after 5 *tm*. The dotted lines are constructions to determine the infusion time (10 minutes) for the amount of indicator (*d*) which passes at 280 cm during 20 minutes (from the 80th to the 100th minutes). The rate of flow of the indicator at this level is thus decreased by 50 percent; *a* represents the amount of indicator which has left the infusion site but has not yet had its transit to the sampling site, i.e., is retained in the first 70 cm segment of the intestine. The amount of indicator contained in each 70 cm segment at different times is schematically presented in the lower part of the figure, where the scale has been reduced by half. The same type of graphic illustration may be used for any assumed relation between linear velocity and distance of intestine passed.

half of Fig 2. As in the fluoroscopic study by Mattsson, the marker spreads rapidly through the intestine during the first 20 minutes after intake of the G-meal and reaches the level of 350 cm in about 1.5 hours; 350 cm was the average length of the intestine from the pyloric to the ileocecal valve found by means of an intubation technique with a tube of the same diameter as in the present study (28).

Since the flow of single indicators at the jejunal sampling site is known, the transit of single indicators through the intestine may be calculated with the same assumptions as stated above. In this way the increasing mixing of the indicators during passage through the intestine can be visualized (Fig 3). The even rate with which the total indicator is delivered at 350 cm suggests that the volume changes per 20 minutes are smaller in lower than in higher segments of the intestine.

Pancreatic enzymes

The estimation of the output of enzymes is jeopardized by several factors. Proper handling of samples after the collection does not exclude inactivation during the transit of the intestine and of the tube. Amylase is rapidly inactivated under acid conditions but otherwise stable. Lipase is less sensitive to acid reaction and fairly stable in the pH range of obtained samples. Nevertheless, preliminary tests have indicated that inactivations up to 25 percent could be present during transit. The trypsin values have in our experiments shown the greatest variations. The complicated interactions between activation of trypsinogen and inactivation of active trypsin, as well as a less suitable method involving precipitation of the enzyme, may have contributed.

The condition mentioned above has made it futile to analyze the relation between enzymes in detail. Observed data indicate that in our study (as in

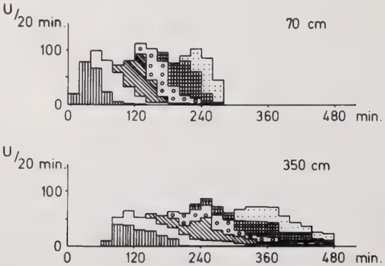

FIG. 3. Comparison of the passage of the different intraduodenally infused indicators at the sampling site in the jejunum with their expected behaviour at a distance of 350 cm from the infusion site. Means of the single indicator amounts which passed at the investigated level per 20 minutes in the seven G experiments were used in the calculations.

other studies) the quotient between different enzymes varies much between, but little within, individuals.

As in all measurements of enzyme output from intestinal samples an error is introduced by the enzyme contents in the "dead space" between the secretion and sampling site. In the secretin tests of Lagerlöf the amylase contents of the duodenal loop were estimated to about 200 amylase units. Corresponding figures for 70 cm could then be up to 400 units, which is 25 to 50 percent of the obtained amylase output during the first hour. This amount and corresponding amounts of other enzymes evidently constitute a preformed pool ready to be mixed with the first portions of emptied gastric contents. If this pool is included, significant correlations are obtained between the amounts of lipase passing duodenum and the simultaneously emptied total calories (Table I, Figure 4).

The different time courses of the enzyme output after the different meals invite to speculations concerning their cause. For reasons given, regarding

TABLE I

A pooled correlation coefficient (r) *for the relation between emptied calories (kcal/20 min) and output of lipase (L.U./20 min) was calculated for each test meal from linear regressions in the separate experiments. Probability* (p)

	r	*p*
NG	0.716	<0.10
G	0.572	<0.05
GP	0.758	<0.05
15%	0.808	<0.05

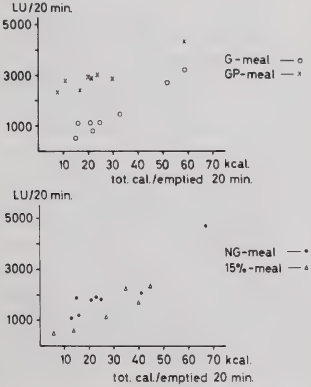

FIG. 4. Relation between the average emptied calories (kcal/20 min) and average output of lipase (L.U./20 min) after the different meals.

gastric secretion the central vagal stimulation should be feeble and lasting only a short time whereas cholecystokinin or cholecystokinin-like hormones and reflex vagal impulses would be the main operators after all meals. Young and Chisholm (23) have shown a short-lasting cholecystokinin liberation to the blood after hyperosmolar glucose solution, reaching a maximum after about 15 minutes and then rapidly declining. A similar peak would be responsible for the initial rise in the lipase output after our 15 percent glucose solution. The approximately linear decline of lipase output in following periods is of the same order of magnitude as found by us in experiments with single cholecystokinin injections and is in accordance with an assumption of exponential fall of obtained serum cholecystokinin concentration and an exponential dose-response relation.

The other three meals contain fat and protein, long known to stimulate enzyme secretion.

The strongest stimulation occurred after the GP-meal, after which the emptying of protein and also of total calories was highest. The output of amylase after this meal was of the same order of magnitude as that found by Schütz, Andersson and Lagerlöf after continuous submaximal stimulation with secretin and cholecystokinin (26).

The output of bilirubin indicated gall bladder contraction after all meals. Since cholecystokinin and vagal reflexes should be the main operators both in enzyme production and gallbladder emptying, the similarity of the response pattern of both is not surprising.

Intestinal absorption

All gastrointestinal events after a meal have a common goal: to contribute to a useful distribution of absorption in time and space. All changes of gastric emptying, duodenal secretion and intestinal transit time will therefore affect time and site of absorption. The general concept that absorption of sugar, protein and fat are completed in the upper jejunum, which stems from the classical work of Borgström et al (29) must, therefore, be modified when the present technique offers opportunities to study the relation between absorption and the variables mentioned.

In the normal gut the absorption of food constituents per time unit is mainly governed by two measurable factors—load per time unit and the time available for absorption, the transit time. At a given mean transit time an increased load leads to a proportional increase of the absorption indicating maximal absorption to take place in a greater part of the investigated segment. At the same time the part of load absorbed decreases. A decrease of the mean transit time will have the same effect as an increased load.

During the first time after the ingestion of the meal the load is high and the mean transit time short. A considerable portion of the emptied food constituents are therefore propelled to lower parts of the intestine. By the increased area available the absorption per time unit is thereby increased. At the same time a great part of the intestinal neurohormonal apparatus is activated, resulting in gallbladder emptying and rapidly increasing water and enzyme secretion from pancreas. To these events may be added the building up of an increased motility, the main function of which is mixing and resistance to flow, both important factors for the ensuing absorption.

During later phases after the ingestion of a meal, the load is usually relatively small and the mean transit time high. Food constituents emptied from the stomach are therefore absorbed at higher levels.

The findings may be extrapolated to more voluminous meals which, according to Hunt, may empty more rapidly. The intestinal load will be higher and the mean transit time, as a result of the increased load, will be shorter. More unabsorbed material will reach lower parts of the intestine. In this way, big eaters find use for their longer intestines (30).

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